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THE APPLICATION OF EXPERIMENT TO THE STUDY OF CANCER

By E. F. BASHFORD, M.D.

General Superintendent of Research and Director of the Laboratory of the Imperial Cancer Research Fund

Dogmatic statements regarding the nature of cancer have broken more reputations than they have made. Paradoxical as it may be, progress in our knowledge of cancer has been made mainly by failure, by successive generations of investigators proving the insufficiency of the knowledge and the hypotheses of their predecessors; but this applies equally to the advancement of knowledge in many fields. The advancement of knowledge by the process of exclusion is surely the most tedious of methods, since, beyond all else, it signifies inability to formulate correctly the problem which it is sought to solve. We are still struggling to formulate correctly the problem, or better, problems of cancer, the sum of our facts not yet sufficing for an unequivocal statement of a problem which shall finally rid us of the everlasting question, What is the cause of cancer? We must be patient of the necessity for undertaking destructive work. In the face of the many defeats efforts at constructive work have suffered in the past, we must be mindful that hypotheses and theories, like men, "may rise on stepping-stones of their dead selves to higher things." Finally the truth is elicited. While avoiding dogmatic statements, I hope to show that the application of experiment to the study of cancer is rational to-day, and has advanced in four or five years to a stage where the results have become of constructive value.

In the space of a short article it is impossible to give an adequate account of the many aspects from which "cancer" requires to be studied. I propose to consider mainly the application of the experimental method to the study of growth, and to show how greatly knowledge has advanced since the first experiment on cancer was made in 1773. At that time

experiment was guided by reasoning from entirely erroneous premisses. For a long time subsequently, knowledge was greatly advanced by descriptive investigations undertaken with the assistance of the microscope. Later, experiment was resorted to again, in applying the methods of bacteriology, with results which showed rather what cancer was not, than what cancer was. Biological knowledge required to advance in other fields before the experimental method could be so applied to the study of cancer as to yield positive contributions, carrying us forward from where clinical, histological, and bacteriological studies had reached their limitations, and from where physiology and chemistry could find no point of attack.

The Academy of Sciences, Belles Lettres, and Arts at Lyons proposed the following as the subject of a prize dissertation for the year 1773: "To form such inquiries on the causes of the cancerous virus as may lead us to ascertain its nature and effects, and best methods of obviating it." The foul sloughy discharge, the "cancerous virus," proceeding from ulcerating, septic, "open cancers," was at that time held to be the characteristic of the disease, and the subject selected was the direct incentive to the first experiment on cancer.

The prize was awarded to Bernard Peyrilhe, who remarks, in the course of his essay, "With respect to the contagious nature of this virus, it must be acknowledged that, either externally or internally applied, it is capable of infecting the healthiest of men." . . . He found that by the subcutaneous injection of what he regarded as the "cancerous virus," he could reproduce those clinical features which were at the time supposed to be characteristic of the disease. The correspondence seemed to him, and to the judges of the prize, to be so close as to merit the inference that he had communicated cancer itself, although the experiment terminated prematurely. His essay was ordered to be published by the Academy. It attracted so much attention that it was translated into various languages, and influenced medical opinion for many years. The experiment really bore on the transference of secondary bacterial infections, and not on the transference of cancer. The experimental investigation of cancer is to-day something entirely different. The *sine quâ non* to its satisfactory pursuit is the fulfilment of the requirements insuring absolute asepsis. The animals which are made to bear cancerous growths artificially, suffer little or no incon-

venience; since secondary bacterial infections are inimical to the objects of the experiments, they are carefully avoided. As will be explained below, the transference of cancer differs fundamentally from the transference of any known infective disease.

Peyrilhe's experiment may, however, be held to have been positive, in that he had produced artificially what were then regarded as the particular features of the "cancerous virus." The repetition of Peyrilhe's experiment to-day could not be justified as a means of throwing light on the nature of cancer. Peyrilhe was under the influence of Descartes' lymphatic theory, as were his contemporaries Alexander Monro and John Hunter in this country. He was ignorant of the true structure both of cancer and of the healthy tissues, and the "cancerous virus" itself was nothing more than the consequences of the action of the organisms of putrefaction on the tissues. It was not till one hundred years later that Lister's experiments on wound infections demonstrated conclusively that this was so.

Progress was about to be made along unsuspected lines, and during a century the valuable additions to knowledge of the nature of cancer remained almost entirely descriptive. The developments of normal and pathological anatomy, of histology and of experimental physiology, riveted attention. They yielded, as the end result, criteria of the minute structure and nature of cancer absolutely different from those by which the success of Peyrilhe's experiment had been tested. The "cancerous virus" survived; but had already acquired a changed and vaguer connotation, when, in 1840, Langenbeck found solid nodules—new formations of tissue—develop in the lungs of animals after inoculating them with cancerous *tissue* from a human breast. Those nodules were taken to imply that the animals had become infected from human cancer, as the nodules in Villemin's experiments (1865) were interpreted—as we now know, correctly—to mean the infection of rabbits by the inoculation of tuberculous matter from the human subject.

The significance of the many resemblances of cancerous to healthy tissues was still unappreciated. It was long in being fully comprehended. Its importance received occasional emphasis early in England, *e.g.* by Pott, Abernethy, Wardrop, Astley Cooper, Walshe, Hodgkin, Everard Home, Carswell, Wilks, and others, and was fully recognised by Johannes Müller and his pupil Virchow. In 1868 Wilks emphasised how this similarity

differentiated the disease from infections. Thiersch, Waldeyer, and others finally established the relation of cancer to normal tissues, and its probable origin from them in many cases, in opposition to the humoral theories which found their last exponent in the distinguished Virchow.

Experiments like Langenbeck's were repeated with great perseverance even up to the present decennium; but, with the development of bacteriology, they were modified in many instances, where the experimenter believed he had discovered the "virus" or other minute parasitic cause of the human tumours. Whereas many observers were unable to induce cancer in animals in those ways, others claimed success. Criticism always effectively disposed of those claims. The tumours produced in animals bore vague, or even only imaginary, resemblances to the tissues of the human tumours employed in the experiments. Many were proliferations of the inflamed tissues of the animals themselves, as the result of the experimental inoculations, while others were normal and pathological structures encountered in animals, but not in man, and entirely independent of the experiments.

All this time descriptive pathology and study at the bedside were advancing knowledge, leading ultimately to the accumulation of an overwhelming amount of evidence in favour of conclusions in two directions: (1) the cancerous tissues destroyed life mainly, if not solely, by their powers of progressive proliferation and of dissemination in the body: (2) the proliferation arose primarily in a circumscribed area, and proceeded from causes inherent in the growing cells themselves. It required many years before the early surgical treatment of the disease ousted all other methods, and was acknowledged to be justified absolutely by increasing certainty of the circumscribed origin of cancer. This had been clearly enunciated by Wilks as a result of investigations pursued between 1847 and 1868, and was stoutly defended by Campbell de Morgan and Charles Moore, against the constitutional views of the disease ably expounded by Paget and his followers. The opposing schools joined issue in a memorable debate before the Pathological Society of London in 1874. The methods of descriptive pathology and of clinical study ultimately reached their limitations, for they were inadequate to explain the powers of growth, which they had sufficed to

define as embodying the essential problem of cancer. So distinguished an observer as Campbell de Morgan confessed that "experiment fails us, and we know nothing of the earliest stages of the disease." The search for a specific cancer cell proved futile, according to Wilks, as early as 1868. Investigation of the minute intra-cellular structures provoked much controversy during the last twenty years of the nineteenth century, but remained, as it remains to-day, barren of positive results. Discussion of the infective or non-infective nature of cancer became entirely hypothetical. All attempts to induce cancer in animals by inoculation from the human subject having led to negative results, the method had been practically abandoned. Efforts to elicit experimentally the hypothetical (latent) powers of growth of embryonic tissue in support of Cohnheim's hypothesis, or those of groups of adult cells whose "organic continuity" with the tissues was dissolved, as postulated by Ribbert, showed how limited were the powers of growth of embryonic and adult tissue. In the case of embryonic tissues they showed also that the ultimate cessation of growth was accompanied by a differentiation into tissue of adult type.

The acquisition of new knowledge seemed very remote indeed. In spite of the most discouraging outlook, courageous and serious resort to experiment had become a pressing necessity at the end of the nineteenth century, and, fortunately, the success attending subsequent experiments has conferred a positive value on much of the apparently negative experimentation described above.

The study of the phenomena called forth by inoculating the tissues of animals into others of the same, and of alien species, showed during the closing years of the last century how distinct —chemically and biologically—the tissues of one species of animal are from those of another, even when nearly allied and elaborating their tissues from identical pabulum. The demonstration of the difference appears to have re-directed attention to a few earlier experiments on the transmission of tumours from one animal to others of the same species. Statements of the success attending the deliberate inoculation of human beings with portions of their own cancerous tumours were remembered. Observations made by Hanau, Jenny, Morau were remembered as indicating the transference of the disease from rats and mice to others of the same species.

It was by no means clear how those experiments were to be

interpreted, nor how the disease had been transmitted, and they had been received with scepticism apparently justified by repeated failures to transmit human cancer to animals. The recollection of them was born of the despairing hope that, perhaps, after all, statements relating to cancer in animals had been unduly neglected. It had been asserted frequently that the disease was peculiar to man, even to civilised man, that it was rare in other races of mankind, and in the domesticated animals with whom he came in contact, but altogether absent in aboriginal races and in wild animals. It is only fair to add in passing that veterinarians had described all the main types of cancer in the domesticated mammals.

At the time of the foundation of the Imperial Cancer Research Fund the position was briefly as follows: The gradual exhaustion of the methods in vogue had led to a cessation in the advancement of knowledge on the nature of malignant new growths. The bedside observation of cases in the human subject, combined with anatomical, microscopical, and chemical examinations in laboratories attached to hospitals, had advanced to a stage where they seemed to raise more difficulties than they solved. As in the corresponding stages of previous attempts to solve other anatomical and physiological problems, the number of contradictory speculations current was legion. They were based on the same observed facts, which were being added to continually by other facts of the same kind—*i.e.* there was vain repetition. The exact knowledge of the disease was strangely restricted to its occurrence in the more civilised races of man, while the explanations of the observed facts roamed over the full field of medical and biological knowledge, and speculation. This discrepancy required adjusting. The impossibility of directing speculation into proper channels, or even of determining what those channels might be, by other methods, caused the need for comparative studies and for experiment to be acutely felt.

The first organised attempt to broaden the basis of the knowledge of cancer, and of its experimental study, was made when the investigations of the Imperial Cancer Research Fund [1]

[1] A "Society for Investigating the Nature of Cancer" was formed at John Hunter's instigation in 1804. The German Cancer Committee was founded in 1900; its work was, however, directed to applying statistical methods to prove the infective nature of cancer. The New York State Cancer Laboratory at Buffalo was in working order in 1899, the work has been almost entirely experimental; but mainly directed to search for evidence in favour of infection.

took definite shape on the basis of a scheme of research submitted to the Executive Committee by myself in October, 1902, and diligent search was instituted into the occurrence of cancer not only in all races of mankind, civilised and aboriginal, but also throughout the animal kingdom. A farm was secured for observations on the larger domesticated animals suffering naturally from cancer, and every opportunity was utilised of testing the possibility of transferring the disease and studying it under experimental conditions. The scheme of experimental research, thus embarked on, received an unexpected justification and a great impetus by the publication of Jensen's accurate and fundamental observations on the inoculation of a tumour of a mouse in 1903, and contemporaneously of those of Borrel. Since then valuable work has also been done by Apolant, Haaland, Loeb, Michaelis, Henke, and, above all, by Ehrlich.

Jensen's great service consists in the fact that he did not restrict himself to demonstrating the reproduction at the site of inoculation of the features of the original growth. He also proved,[1] by carefully following the processes at the site of inoculation step by step, that the new formation was due to the continued growth of the cells peculiar to the tumour, parts of which he had introduced. Jensen's work has been energetically followed up in all civilised countries, and in the first instance in the laboratory of the Imperial Cancer Research Fund.

The investigations of Murray and myself on an adenocarcinoma mammæ and Jensen's own tumour confirmed his observations, and, by extending them to a large number of other malignant new growths in mice, established the experimental study of cancer on a secure foundation; so that, after some preliminary controversy, it is now universally agreed, infection plays no part in the process of experimental transference. On the contrary, transference to fresh mice merely affords the cancer cell an opportunity for continuing to proliferate in fresh soil. With Murray I made an exhaustive study of the processes at the site of the inoculation of cancerous tissue in the case of

[1] This demonstration is sometimes erroneously attributed to L. Loeb, who obtained tumours on inoculating sarcoma in rats in 1901-3. Loeb's original description of the processes at the site of inoculation does not bear this interpretation. He also admitted the possibility of the tissues surrounding the grafts acquiring sarcomatous properties.

twenty sporadic mouse tumours, and conclusively proved that the cancer cells are merely transplanted into the new animals which provide them with nourishment, by means of a specific connective tissue reaction supplied afresh after each fresh transplantation.

The criticism, that the tumours experimented with were not comparable with cancer in the human subject, was advanced as speedily as of yore, and with all the assurance born of a knowledge of its effectiveness in discrediting earlier experiments. It is still feebly uttered by certain persons who lack extensive experience of cancer in animals. At last, however, it was to be met satisfactorily. Before proceeding to the study of other problems, Murray and myself spent much time in demonstrating, not only that the starting-point of the experiments has been cancer of the mamma, but were at great pains to reproduce artificially metastases, infiltrative and expansive growth—in short, all the main features of the disease in previously healthy animals. With Cramer we drew a parallel between the growth of cancer under natural and experimental conditions. Haaland independently did the same for a number of carcinomata, and demonstrated that Ehrlich's experimental sarcoma formed metastases, and behaved in other ways like a malignant new growth of the human subject. Apolant published an elaborate and valuable monograph on the histology and pathology of the mammary tumours of the mouse.

These results are based on observations all but limited to the mouse. As yet our numerous inoculations of cancer in other vertebrates have met with only transitory success in rats and in a single carcinoma mammæ of the dog. Even in the case of mice there are great difficulties to be overcome: for the other vertebrates many of the difficulties are still unsurmounted. The failures may be due largely to the relatively small number of inoculations made as contrasted with mice, as well as to difficulties associated with varying suitability of the animals employed, which the study of the transplantation of cancer in mice has proved is a factor of great importance.

The inoculation of cancer in mice has been necessary on a scale perhaps unprecedented in the experimental study of disease, in order to prove that the cancer cell is able to grow and to proliferate continuously in animals of the same species only. To establish that the cancer cell is not transferred

naturally from one individual to another, equally extensive observations have been necessary. As I shall show later, the cancer cell of the mouse is in all probability a mouse cell incapable of nourishing itself and proliferating, except when in intimate organic union with the body, and nutrient supply, of the living mouse. Ehrlich has shown that a transitory growth follows the transplantation of one of his mouse tumours into rats. We found that the same power is possessed by other mouse tumours. Growth, however, speedily ceases in rats, although it is retained unimpaired if the cells be re-transferred to mice after a short sojourn of six to eight days in the rat's body. A mouse tumour does not grow equally well in all mice. We found difficulty at first in getting Jensen's Danish tumour to grow in English mice, and Michaelis and others have entirely failed to get it to grow in the mice of Germany, England, and other countries.

The progress made by the demonstration, that cancer could be transplanted and artificially propagated *ad libitum*, was not of the obvious kind appealing to the multitude. If transference had proved that the tissues of the new animal acquired cancerous properties, *i.e.* that the disease could be conveyed by way of infection, our knowledge of its nature would have been advanced enormously at one step. Then investigation could have been legitimately limited at once to identification of the agent and of the channels of infection.

The nature of the transference of cancer was out of accord with all known processes of infection, in this respect merely presenting old problems in a new light, as well as new problems for solution. However, Murray and myself were able to draw certain conclusions other than those drawn by Jensen, which have been abundantly confirmed since by others: (1). The amount of proliferation exhibited is enormous, once the primary difficulty of transplanting a mouse tumour at all has been overcome. The proliferation is then out of all conformity with the laws of growth in vertebrate organisms. The cancerous tissue retains its histological characters. (2) If a large number of healthy mice be considered, growth proceeds readily in mice in which cancer is rare naturally. Although the maximum incidence of cancer occurs in the later years of life, growth proceeds as well, and even better, in young animals than in old. Therefore, if senescence is intimately bound up with the

origin of cancer, it is unnecessary for growth to continue. (3) The conditions of the origin of cancer were to be sharply distinguished from those of continued growth. (4) The progressive, apparently vegetative, growth was to be explained as inherent in the cancer cells without the assumption of the stimulus of extraneous agents. The amount of growth presented a cell problem of importance in itself and required to be further analysed. (5) The purely cell problems of cancer required to be attacked by the methods of experimental and comparative biology rather than from the narrower standpoint of human pathology.

Those conclusions would have been illegitimate if drawn only from experiment at that time. They were advanced also on the basis of a comparative study of the disease in the vertebrates down to marine teleostean fishes living in a state of nature, and after careful statistical studies of the national mortality from cancer, and of its occurrence in the patients of London hospitals. The zoological distribution of cancer made it obvious that it developed in man, independently of any direct influence proceeding from civilisation, diet, or other one of many inconstant external factors. Subsequent studies in the same direction, and also into the ethnological distribution of the disease, have but rendered this inference more justified. The disease has been found in all those races of mankind among whom diligent search has been made. Where it was said to be rare it has been found to be common—*e.g.* in Japan some 25,000 deaths are annually recorded from cancer. Where it was said to be absent it certainly occurs. Of course, no comparison or statement of relative frequency is possible between civilised and aboriginal man, and between tame and wild animals. The fact of prime importance is that the disease is of universal occurrence, pervading all forms of vertebrate life, and everywhere adapting itself to the conditions presented by different species. The disease is universal, but the difficulty of transferring it even to individuals of the same species is unique, and when successful is not to be confounded with the production of cancer *ab initio*.

Throughout the entire distribution of the disease two facts stand out prominently: The number of cases recorded is in direct proportion to the care with which it is possible to make examinations, and to the number of adult and aged individuals

examined. That cancer develops with increasing frequency with advancing years in all animals, whether their lives be long as in the case of man, or limited to two or three years as in the case of the mouse, is a biological law of universal application. The only class in which cancer has not yet been found, viz. the reptilia, is the exception proving the rule, for in them life may extend to hundreds of years, and the opportunity for examining a sufficiently large number of aged reptiles does not exist. The same applies, if in lesser degree, to the apparent rarity of the disease in wild animals generally, since they do not naturally survive the functional activity of their teeth or their reproductive system.

The greater recorded frequency of cancer in domesticated mammals as compared with wild animals implies, not that the disease is communicated from man, but simply, that man protects them and provides for them, so as to permit of their attaining their respective cancer ages.

In the case of the organs of the human body, cancer exhibits a corresponding dependence on the duration of life, *e.g.* chorion epithelioma develops before birth and presents a perfect parallel to the incidence of cancer in short-lived animals. In a general way it may be said, since space prevents my discussing details, other organs present all gradations up to the skin, which is functional so long as life lasts, and in which the maximum incidence of cancer is only attained in extreme old age. The age of the individual organism is of less moment than the senescence of its several tissues in determining the incidence of cancer. Hence cancer may occur at any age, in the new-born, and even before birth. As a matter of fact it is more frequent at birth than between the tenth and fifteenth years of life. It is therefore wrong to assert that cancer is caused by "old age."

The generalisation as to the constant association of the incidence of cancer with senescence adds greatly to the significance of its unbounded proliferation, when propagated by transference from one set of mice to another *ad infinitum*, since the size of the body and of its individual organs, as well as the length of life, are specific for each species of animal. They distinguish different species as sharply as any of the other criteria on which the systematic zoologist depends for his classification, *e.g.* as sharply between the mouse and the rat as between the mouse and the elephant. As I shall describe below,

cancer frequently stops growing, and this is the most encouraging result obtained by experiment. Nevertheless, there is as yet little reason to suppose that limits corresponding to those set to the growth of organisms and their organs, are necessarily set to the growth of cancer in any of the vertebrates, except when artificially set by the death of the animal in which it originates or is growing. The primary transplantations of our sporadic tumours have succeeded for 66 per cent., and ultimately all may be found to be transplantable. Hence, since a mouse tumour has now been growing twice as long as a mouse lives, the more limited evidence of a similar phenomenon in other vertebrates, *e.g.* rats and dogs, is probably due mainly to insufficient experimentation, and to failure to supply suitable soil. On the one hand, we have the limited powers of proliferation of normal tissues which never produce tumours when transplanted, and the unlimited proliferation of cancerous tissue; on the other hand, we have the dependence of the inception of cancerous change on senescence of the tissues as determined by the laws limiting the duration of life specifically. We have stated that in this association lies the crux of the problem of cancer, and R. Hertwig in referring to physiological senescence, and others, have also noted its biological importance. This association involves a problem which cannot be attacked directly at present, because we are still unable to determine the inception of cancer experimentally. We can, however, approach it indirectly by the prolonged study of the growth of cancer when once it has started, and endeavour to determine if it be purely vegetative, dependent entirely on the supply of adequate and suitable food, or be perhaps also maintained and renewed by some intra-cellular reorganisation occurring periodically, at a time when growth tends to cease naturally. In short, we must find out if the study of growth gives us any clue to its nature and origin. We can also study the conditions favouring and retarding its continuation or modifying it. Thus, the experimental study of cancer is at present essentially the experimental study of the growth of cancer; but this is no subsidiary attribute—it is in reality that property of cancer by virtue of which it destroys human life. It is customary to consider the growth of tumours as expansive or "benign," and infiltrative or "malignant," a classification corresponding also to their clinical behaviour. As a matter of fact

the two forms of growth are interchangeable experimentally. For different carcinomata Murray, Cramer, and myself have shown that this interchange of the mode of growth is dependent on the anatomical surroundings of the tumour, and not on an alteration of the properties inherent in the tumour cells. It therefore suffices to study mere growth, and in doing so one may disregard the features of most use in the current classification of tumours.

The transplantation of a sporadic tumour is effected according to our method as follows: The tumour is removed from the animal so as to avoid bacterial contamination, and weighed. It is divided into portions which are placed in separate receptacles. The portions are then inoculated by means of hypodermic needles, minute fragments (*circa* 0·01—0·03 grm.) being broken off and inserted subcutaneously. The tumour is thus distributed over as large a number of mice as possible—it may be fifty or four hundred. The experimenter must then possess his soul in patience. Rarely is he rewarded by the appearance of true tumours, at the site of inoculation, within a fortnight. He may have to wait from three to six months before the inoculations can be pronounced to have fallen out either positively or negatively.

The nature of the result is determined mainly by two factors: variations in the suitability of the soil the mice afford for the growth of the grafts introduced as above; and variations in the character of the tumour cells, not only of different tumours, but of one and the same tumour. The suitability of the soil may be taken to be fairly constant when large numbers of young mice of the same age (six to eight weeks) and of the same stock are employed. Adult and old mice are not only much less suitable than young animals, but they also exhibit greater individual idiosyncrasies. Normal mice may be said to offer a certain unsuitability, or to be resistant, to inoculation, when tumour cells are transferred to them after removal from their natural environment in the animal in which they developed. This resistance acts as a sieve, sifting out the tumour cells which are unable to nourish themselves and proliferate in this new and, it may be, very strange environment. Thus the positive results of the primary transplantation seldom form a high proportion of the animals inoculated. They are probably obtained by the segregation of groups of cells of high assimilative energy and

growth, in mice whose resistance is below the average, or rather the suitability of whose soil is above the average. A summary of some observations we have already published will serve as an illustration. The final results of transplanting 32 spontaneous tumours of the mamma show that 2,278 of the mice inoculated remained alive three weeks later, and 72 of them ultimately developed tumours—*i.e.* only 1 inoculation out of 31 was successful, or 3·2 per cent. A tumour is propagated by a repetition of this process of transplantation from one series of mice to another. But the subsequent transplantations are only exceptionally accompanied by better success. In illustration of the pertinacity necessary in such experiments, I may state that it was not till we had repeated the primary process above described for twenty-seven different sporadic tumours, and also repeated it for each tumour one, two, three, and four or more times, that we alighted on a growth capable of unlimited propagation. This difficulty in starting propagation illustrates merely one aspect of the initial obstacles which those who embarked on the experimental study of cancer have had to overcome in the case of mice, and are still striving to overcome in the case of other animals.

When once a tumour has been got to grow well in mice of a particular race, similar difficulties are often encountered in transferring it to mice of strange race. Thus we added the study of Jensen's tumour, which was accustomed to Danish mice, to those discovered by ourselves with the greatest difficulty. Michaelis and other investigators have failed altogether to get Jensen's tumour to grow in strange mice. We failed also to propagate a tumour sent from Paris by Borrel. Our experience leads me to surmise that those absolute failures may have been due partly to an unfavourable phase of growth of the cells of the tumours. Our initial success with Jensen's tumour was only $4\frac{1}{2}$ per cent. of the inoculations.[1] When the tumours grown in English mice were transplanted again into English mice, the percentage soon equalled that obtained by Jensen in Danish mice, and in single experiments greatly exceeded it,

[1] The tumour had been removed from a mouse, was forwarded by ordinary post hermetically sealed in a glass tube, and transplanted into English mice five days later. Our primary transplantations were therefore made with an added difficulty, but they also illustrated the vitality of the tumour cells after separation from the body.

rising to 90 per cent. at the third transference. When once the initial difficulties have been overcome, the growth exhibited by propagated cancer, as illustrated by several of the tumours we have studied, soon passes beyond the bounds of all measurement. A small piece of tissue weighing no more than 0·01 grm. frequently increases to 1·5 grm. of tissue in ten days. This is an increase of over a hundredfold, and under suitable conditions 90 to 100 per cent. of the animals develop tumours through many successive transplantations. This sequence of events is not constant when tumours can be transplanted successfully.

Some sporadic growths grow well from the outset of propagation. For others a marked increase in the success of transplantation is observed, frequently after three or four transferences. For others again the percentage of successful inoculations cannot be raised to, or maintained at, 100 per cent.; but remains constant within certain fluctuations. However, for the majority of our carcinomata growth ceased at the third or fourth transference. We have interpreted those differences to signify primary differences in degree, inherent in the cells of different tumours. They persist during continued propagation in the majority of cases; so that each tumour exhibits from the outset of propagation a constant behaviour within limits. We have refrained from terming the increased success of transplantation occurring in special cases "an increase of virulence," as has been done by analogy with what occurs when bacteria are propagated by the method of *passage* from one animal to another. The term "virulence" should be dropped and "adaptability" substituted, because the method of propagation is essentially an artificial selection of the most rapidly growing tumours, and their subdivision and distribution over a large number of animals. Although the greater success sometimes attending later inoculations suggests an alteration in the energy and rate of growth of the tumour cells, the phenomenon referred to may be explained without having recourse to this assumption: (1) by an increase in the number of proliferating cells, similar to what occurs in the propagation of domesticated animals as in the following example; and (2) by adaptation.

The increase in the numbers of any form of fancy dog which happens to become fashionable is due not to an artificial shortening of the period of canine gestation, but to increased breeding facilities, *e.g.* to multiplication of the number of those

who find it profitable to breed that kind of dog. There is a multiplication of the number of dogs breeding, so in the artificial propagation of cancer we multiply the number of cells proliferating. I discuss later the possibility of biological alterations leading to increased rapidity of cell growth. Propagation segregates in the first instance the cells which can accommodate themselves to the artificial conditions, and then provides for their nutrition and proliferation. Thus there are more of the suitable cells in each of the later grafts, and within a given period, this primary advantage gives bigger tumours from each single graft, and a higher percentage of tumours over the total number of animals inoculated.

A violent change in environment is effected when a tumour is removed from the animal in which it developed. The manner in which the change is effected is important. Whereas we have succeeded in transplanting 66 per cent. of the sporadic growths we have discovered, Ehrlich has transplanted 14 per cent. of his tumours successfully. The discrepancy is certainly due to the difference in method. As a rule only the tumours best suited to propagation survive Ehrlich's procedure, while we have obtained a broader basis for the study of the growth of tumours, since our material includes tumours of all degrees of transplantability. One group of tumours does not survive the process at all. A second group grows for a variable time and then dies, being unable to adapt themselves to a strange and presumably an unfavourable environment. The tumours of a third group are able to adapt themselves gradually. The fourth group adapt themselves quickly or at once, and this is the small group which are easily propagated. It is possible that among them there are tumours surviving transplantation, not only because of the inherent properties of their cells, and their capacity for adaptation, but because the change in environment is in reality to a more favourable soil. In the third and fourth groups there must be also more and more cells suited to propagation in the grafts at each additional transference, till a maximum is reached. Although the group of tumours giving maximal success on propagation are of practical importance in experiment, because of the ease with which they permit of controls to attempts to modify growth, their theoretical value depends on their relation to the varied degrees of energy of growth exhibited by the whole group of tumours. It is

obviously of importance to determine experimentally whether or not all tumours tend to augment their energy of growth to the maximum exhibited by this group, and at the same time to assume the same histological garb. At present many tumours appear to retain their own peculiarities, and although the energy of growth may increase, it is not yet possible to determine whether or not the tumours of one organ ultimately approximate to one form when growth is prolonged sufficiently.

I may point out here that the first and second groups of tumours behave on transplantation as embryonic and adult tissues do, by exhibiting limited powers of proliferation, with the difference between cancer and embryonic tissue, that the former does not differentiate into an adult form. We are therefore able to place a positive value on the negative efforts which have been made to produce tumours by propagating normal tissues, and we are able to establish all gradations from the limited growth of normal tissues under experimental conditions, up to the remarkable phenomenon exhibited by the unlimited growth of the cells of some cancers under the same conditions. Thus experiment has bridged over the gulf between normal and cancerous tissue so far as their powers of growth are concerned. The limited growth of normal tissues, when transplanted, is independent of any extraneous organism. There is no need to assume such a dependence when cancerous tissues exhibit corresponding limitations; and if not, why should it be necessary to assume this intervention when the powers of growth pass insensibly through all gradations to those of unlimited amount?

The possibility of an accelerated *rate* of proliferation may be entertained when a tumour has been continually transplanted during long periods, as in the case of Jensen's tumour. If I understand Ehrlich rightly, this is what he means when he states that the increase in "virulence" is due to the avidity of the receptors having attained a maximum in consequence of rapid passage from animal to animal. I shall show immediately that there are natural fluctuations in the amount of proliferation. In the case of rapidly growing tumours all the inoculations which are successful yield tumours quickly (within, say, ten days). Mice then negative remain so. The fact shows that rapidity of proliferation is closely bound up with an extreme susceptibility to nutritive requirements. Since growth presents

natural variations, an increase in its rate may be one result of our artificial selection in long-continued propagation. In passing I may merely mention it is now well established that the amount of proliferation can be influenced in the opposite direction—viz. diminished by exposure of a tumour to heat. The environment may be of great importance when a tumour is propagated for years. Theoretically the long-continued growth of cancer cells in the soil provided by the mice of one stock or country may handicap them for growth in the different soil provided by mice of another; but, whether this be so or not, augmented adaptation to an accustomed soil may result in an enormously increased proliferation. In this direction therefore the cells of later generations may be biologically very different from those remotely antecedent. What occurs when a tumour is propagated by transference through many generations of mice is determined not so much by the number of transferences, as by the long duration of the particular environment, breeding as it were a cell of particular quality. If one follow the process of artificial propagation back step by step to the primary animal, there is no reason why one should stop there, since the gulf between the growth of cancerous tissue and normal tissue after transplantation has been bridged over. What takes place in artificial propagation may well be but an artificial reproduction of what had long been going on naturally in the animal in which cancer developed. However, it is inadvisable to pursue such speculations further at present, for the conditions of the growth of cancer in the spontaneously attacked may be different from those in normal animals, although the lesions are the same. At any rate, the differences between individuals may be of prime importance in determining the nature of growth in the primary transplantation as well as in the individual spontaneously attacked. It seems, at least, that the preceding considerations give us a deeper insight into the nature and clinical behaviour of the cancers of man during their continued growth, and when they exhibit what appears to be a change to a more malignant condition.

The phenomena of growth can all be explained without the assumption of microbic interference. And this brings me to the further consideration of some matters of clinical importance. When the growth of a tumour under artificial propagation is

contemplated as a whole, fluctuations in the rate and amount of growth are observed, even when many batches of the inoculations yield 90 to 100 per cent. of tumours. These fluctuations can be considered from two standpoints: either they are due to variations in the suitability of the mice inoculated, or to others inherent in the tumour cells. While variations in the suitability of the mice, or even of the same mouse at different times, no doubt contribute to their production, they are insufficient to explain all the phenomena. The fluctuations may be revealed by (1) variations in the success of transplantation, (2) a contrast between the sizes of the tumours in different batches, or even in the same batch, (3) a tumour which is growing rapidly may come to a standstill, diminish in size, subsequently grow rapidly, and again diminish, (4) diminution in size, however, is frequently followed by entire disappearance and absorption of the tumour. Those features of growth can be studied in parallel experiments in such a way as to bring out the probability that they are for the most part manifestations of a variation in the assimilative energy of the tumour cells themselves. In the human subject there are corresponding fluctuations in the growth of cancer. In one part of a tumour growth is proceeding rapidly, in another part slowly. Periods of exacerbation alternate with periods of amelioration. Further, secondary nodules of growth are known to disappear while others are growing, and occasionally primary growths have disappeared. The observations made on mice emphasise the importance of those clinical features, largely discredited, in the human subject, since they have been often interpreted to mean that the growths exhibiting them were not cancer. The experimental propagation of cancer has demonstrated conclusively that mice can recover from artificial tumours. In the elucidation of this process, and of its counterpart in spontaneous cancer, lies the hope of assisting the surgeon. At present I can only foresee a still remote possibility that dissemination from the primary focus may be hindered; I can foresee no probability that the primary focus will be got rid of by other means than early surgical removal.

The ultimate absorption of transplanted tumours requires to be considered in relation to certain other facts. The mice in which it has taken place are protected highly against subsequent inoculation with the same growth, and to a lesser extent against

other growths. There is thus a degree of protection which is common, and a certain degree which is specific. The spontaneous disappearance of tumours occurs, entirely beyond our control as yet. But the protection it confers can be imitated artificially. The same degree of common protection can be conferred by the preceding inoculation of tumour material if followed by no growth, *i.e.* if absorbed, and this is not so remarkable as the fact that it can be induced also by the injection of the normal tissues of the mouse and most readily by normal blood ; but not by the cancers or tissues of alien species. The far-reaching significance of the protection which can be induced by carcinomatous, by sarcomatous, or by normal tissues, causes me to emphasise that our observations and Ehrlich's are in essential agreement. We require to learn whether the protection thus conferred is actively induced or only passively conferred. In the case of blood this question was most easily settled. The protection is not passively conferred by the serum, but is actively induced by the blood cells. To sum up, we are able to so modify the soil provided by mice that cancer cannot grow in it. For indications of the change we are dependent on the behaviour of the living cancer cell in the living mouse; we are dependent on experiment absolutely.

Jensen recorded the disappearance of tumours in 1902-3. The first occasion on which we noticed a large transplanted tumour disappear was while studying a batch of mice with his tumour in August 1904. This tumour was exhaustively studied, and the important part phagocytosis played in the process discovered, not only in the case of this entire tumour, but in more limited areas of a very large number of tumours. We have since recorded the same process for other carcinomata, and its importance has been emphasised by others, especially by Clowes. We have observed the same process in localised areas of spontaneous tumours. There can therefore be no doubt that the animal primarily attacked attempts to protect itself by the same means as a normal animal after successful inoculation.

The investigation of the nature of protection is bound up with very great difficulties, which are not diminished by the absence of any analogy with what we know of immunity to infective diseases. Up to the present I have spoken of the propagation of the tumours as if it meant a mere culture *in vivo* of the cancer cells. This, however, is only part of the truth.

As I pointed out on a previous page, the cancer cells acquire at each transference a specific stroma, the connective tissue reaction they call forth in the host. A culture of isolated cells has not been obtained, since growth always proceeds in the form of a tissue with intimate vascular supply. We emphasised the distinction thus revealed between the cells of different growths nearly allied histogenetically, and the parallel it presented to the biological reactions whereby blood relationship has been established. At the same time we pointed out its far greater delicacy. In what I have briefly outlined with regard to protection the same delicacy, or specificity, appears. The cancer cell is dependent on the provision by the host of a connective tissue and vascular reaction, if it is to grow into a tumour. It would appear to be a piece of short-sighted generosity on the part of the host to supply connective tissues to subserve merely the needs of the greedy cancer cells, and yet this appears to be the relation which subsists when a tumour is growing well. The question arises, is the connective tissue reaction supplied only in response to the needs of the cancer cells, or is it really protective, as in the case of the similar reactions elicited by the tubercle bacillus or other organisms? When a tumour undergoes spontaneous absorption, the connective tissue reaction does become protective, and the acquisition of phagocytic properties by its cellular elements is the main factor in removing the remains of the tumour. Thus under certain conditions the organism is certainly capable of defending itself very actively through reaction to the presence of the cancer cells, and after the process remains protected. The conclusion that phagocytosis is the only agency by which protection is conferred would in my opinion be unwarranted at present. Spontaneous absorption appears to be frequently associated with a phase of depressed growth of the tumour cells themselves. We do not yet know exactly whether the cancer cells require to undergo changes—either spontaneous, or induced from the side of the host—placing them at times at the mercy of the connective tissue which, at other times, appears to be their willing servant. When a graft is introduced into a protected mouse, little, perhaps no, proliferation follows, and the tissue speedily dies. Whether it is killed by the fluids of the body, or simply starved to death, because it is prevented from exercising its chemiotactic influence on the connective

tissues, is a problem too hypothetical for discussion here; it will suffice to say, that interference with the chemiotaxis of the cells of tumours of different kind would explain the phenomena thus far observed, their specific and their common features.

Is the protection to be interpreted as due to some virus contained in the cancer cell? I think not. We have pointed out how mere inhibition of the connective tissue reaction would suffice to prevent growth, since its specificity indicates a delicacy of nutritive requirements on the part of the cancer cell far exceeding that of any other known biological reaction. We found that protection is conferred on the mouse only by mouse tumour, not by the preceding inoculation of the tumours of alien animals even so nearly related as the rat. One form of mouse tumour protects against another. We found that protection is conferred on the mouse by the normal tissues of the mouse and not by those of strange species. There is thus something common to mouse tissue and mouse tumour which is not common to mouse tumour and the tumours of other animals. The protection cannot therefore be due to the presence in the tumour cells of a virus common to vertebrate cancer. Reviewing our experiments as a whole, I am of the opinion they establish that the cancer cell is really a cell of the mouse organism requiring the same food, the same kind of connective tissue and blood supply, to which it was accustomed in the mouse in which it developed. The only difference it exhibits is a qualitative one, viz. in the powers of growth and assimilation. Fortunately the cancer cell is very much at the mercy of subtle changes in its environment, and it is by taking advantage of this fact that we have been able to prevent its growth in the living mouse.

The study of cancer in man became increasingly difficult in the course of time because of the mere multiplication of facts defying classification. Malignant new growths in man and in animals—even those of any particular organ and histogenetically related—exhibit an extraordinary variety in their histology and clinical behaviour. This variability is made yet more difficult to comprehend by the inconstancy in the behaviour of tumours histologically indistinguishable. Many attempts have been made to group the phenomena, *e.g.* by pushing the histogenetic subdivision of tumours to absurd lengths, by assuming that progressive loss of histological differentiation proceeds *pari*

passu with prolongation of growth (anaplasia), and, for apparently identical tumours, by assuming differences in the soil afforded by different individuals, and also by the several organs of any single individual. At the outset of our investigations, therefore, the growth of cancer under natural and experimental conditions was selected for study, as covering ground common to all the varied manifestations of tumours. The experimental study of the growth of cancer proved the permanence of the histological characters, and revealed that the cells possessed other qualities of a more or less permanent kind, escaping demonstration by other means. At the same time, experiment shed light on the relations subsisting, on the one hand, between the different properties of cells similar morphologically, and on the other hand the soil in which they are situated. Only by experiment has it become possible to study the growth of one tumour in different soils, and, what is equally important, the growth of different tumours in the same soil. In this way the varying clinical and histological features of the cancers of the mouse's mamma, reproducing as they do in miniature all the difficulties encountered in man, have been relegated to subsidiary importance for the present, in favour of the study of the problem of cell assimilation, which, being common to all these tumours, permits us to unite in one general conception all the gradations and mutations exhibited in the growth of cancer. The experimental study of growth has already enabled us to compare tumours together as regards their biological behaviour (clinical behaviour). It has been proved that cancer grows better in young mice than in old; that some mice of the same age afford a more suitable soil than others; that some sites of the body favour growth while others hinder it; that tumours seemingly identical (when examined by the aid of other methods) can be differentiated from one another, since they possess distinct properties by virtue of which they behave differently in the same soil; that one and the same tumour exhibits increased proliferation alternating with diminished proliferation in a similar nutritive environment, and, this being so, the fluctuations in proliferation are possibly expressions of an alternation in the assimilative powers of the cells of the tumours which exhibit them. Experimental propagation further showed that proliferation can be maintained by normal bipolar division and that other forms of cell division are

unimportant. Division being merely the end phenomenon in the growth of the individual cell, what requires study is not the manner in which the cells divide, but the mechanism of cell assimilation and its relation to nutritional environment. These problems have been attacked experimentally by altering the conditions under which cancer is artificially propagated, and, as I have pointed out above, the nutritional environment can be so altered that the cancer cell is unable to grow. The study of the growth of cancer further permits inferences as to the nature of the changes, in cells and in organisms, leading to the inception of the continuous assimilation of which cancer cells are capable.

Murray and myself pointed out the importance of separately considering two factors in studying the growth of cancer—viz. the conditions of origin and the conditions of growth—and we pointed out that artificial propagation enabled us to study the conditions of growth experimentally. Ehrlich has since penetrated more deeply into the relation of these factors by differentiating the primary cell changes in a circumscribed area from the conditions permitting the cells in such an area to grow into a tumour, and by assuming the importance of constitutional conditions favourable to growth. We have pointed out, with reference to carcinoma mammæ in the mouse, that the differences revealed and shown to be retained in artificial propagation indicate primary differences inherent in the cells of different tumours, and that they can be conceived as due either to a variety of causes or to one causative factor acting in varying degree. As our work proceeded, evidence accumulated pointing more and more definitely to the assumption that one primary change occurring in varying degree explained the different behaviour of apparently similar and nearly related tumours; but we are ignorant of how the primary change is elicited. If histological differences be ignored for the present, the only fundamental differences, from the standpoint of growth, are in the energy of assimilation of the cells, and in the chemiotactic influences they exert on the connective tissues of the host which convey the food supply. In accordance with this conception what are abstractly called variations or differences in malignancy between tumours of the same histological type are concretely expressed as differences in rate of growth. The injury to the mouse in which

a transplanted tumour is growing proceeds only from the consequences of growth, and the demands made for nutriment; but as a rule the mice look quite well, and malnutrition progressing to wasting is an occasional and accidental occurrence.

Thus far I have considered only the growth of cells, but we have to deal with the growth of tumours often weighing nearly as much as a mouse. If cell nutrition is so important it may seem strange to say that the mice usually enjoy perfect health. It was necessary to ascertain if the nutritive balance in an animal had been disturbed by the presence of a tumour in any other way than could be indicated by ill-health and wasting. When very young mice are made to bear rapidly growing and large tumours, they often only attain about half the weight of others of corresponding age. When adult animals bear rapidly growing and large tumours they may lose weight, and more than is accounted for by the weight of the tumour. This they speedily make good when the tumours are removed, and the mice then revert to the normal conditions without any detectable sign of their having ever borne tumours. The gastric contents of normal mice after successful inoculation with tumours contain a large excess of HCl during active digestion, as observed by Cramer and myself, and demonstrated by Copeman and Hake, who have analysed the stomach contents of over five hundred mice. This increase in HCl is not to be considered as out of accord with clinical observations in the human subject. There is much evidence, recently added to by the careful investigations of B. Moore, that the HCl is diminished in patients suffering naturally from cancer. I am at present considering the facts on the growth of cancer in *normal* mice. We require more data regarding mice spontaneously affected with cancer. The contradiction between the state of affairs in normal mice artificially made to bear tumours, and patients bearing them naturally, reinforces the warning I have expressed already. The conditions of the growth of cancer are to be sharply distinguished from the conditions of origin, and I have also pointed out that the conditions of growth in mice naturally cancerous, and in normal mice, differ from one another. The contradiction above referred to is probably only apparent, and when it is resolved we may know much more of the deeper significance of the diminution in HCl in those spontaneously

affected with cancer. This increase in the amount of physiologically active hydrochloric acid is to be regarded as a compensatory response to the needs of a normal mouse plus a tumour. No assumption is made in inferring that the increase in hydrochloric acid is required for the digestion of proteids. The increase in hydrochloric acid is in all probability accompanied by increased secretion of pepsin and trypsin, the ferments concerned in proteid digestion, although this is difficult to determine in mice. The increased activity of proteid digestion is easily comprehended. Whereas, in a general way, the combustion of carbohydrates suffices for the output of energy in an animal organism, proteids are essential to the building up of protoplasm, and the building up of protoplasm is proceeding apace in the continual production of new tumour cells.

On a preceding page I have pointed out the specific characters of the proteids which even nearly allied animals reconstruct from identical pabulum, and how the mouse alone supplies the nutritive environment or pabulum suited to the continued growth of mouse cancer. These facts are to be remembered in the present connection, for there is no need to assume that the increase in proteid digestion involves any other divergence from the normal process. No doubt proteid digestion prepares the food-stuffs for absorption into the fluids of the body in the usual way, and for their utilisation by its constituent cells and by the cells of the tumour. So fine an adjustment can only mean that the cancer cells of the mouse and normal mouse cells compete for the products of proteid digestion. In view of what we know of the specificity of these processes of nutrition, on which so much light has been thrown by Starling and Bayliss, this implies that the cells of mouse cancer are mouse cells able to impress their needs for food on the mouse as a whole by their higher assimilative energy. The facts reveal, at any rate, how great are the demands for food which growing tumours make on healthy and young adult animals, and at the same time show that the normal animal responds to these demands at least by increased proteid digestion. These observations are the mere beginnings of the experimental study of the problems of metabolism in cancer, and which are not easy to attack in so small an animal as a mouse, and will be attacked still more profitably in the case of larger animals, *e.g.* the dog. The problems of excretion which may have practical importance

are not capable of easy solution in the mouse. One can use the organism as an indicator of the nutritive needs and metabolic activity of cells, because in studying propagated cancer we have such a large number of tumour cells of the same kind in one animal. Conversely, as I have shown above, we can use the tumour cells as indicators of changes in the animals. So far as these results go they encourage further experimental investigation into the growth of cancer as a problem of cell nutrition, in which the organism as a whole is implicated, although the demand proceeds from a circumscribed area, limited in the first place to a little bit of tissue no bigger than a pin's head at the time of inoculation. It is obvious that this line of inquiry may have ultimately direct bearings on certain aspects of the disease in man; but I must enforce caution in drawing conclusions, and above all warn the layman that I have said nothing in favour of popular views on diet or disordered digestion as causes of cancer.

This is an opportune stage at which to terminate an outline of the firstfruits of the experimental investigation of cancer, to the development of which the Imperial Cancer Research has very materially contributed. In 1905, 137,128 deaths of males occurred in England and Wales above the age of 35; of these 11,908 were due to cancer. In the same year there were 138,477 deaths of females above 35 years of age, of whom 16,875 died of cancer. Therefore of persons dying above the age of 35, one out of every eleven men died of cancer, and one out of every eight women. Is this great frequency as a cause of death to be explained as due to communication of the disease from one person to another? Our experimental investigations have revealed no analogy with any known form of infection. The chance of ultimately dying of cancer is one in eight for women and one in twelve for men above the age of 35. A simple calculation shows the probabilities of the occurrence of one or several cases of cancer in families of whom few or many survive beyond the age of 35. Murray and myself have pointed out that when the full facts are known, the incidence of cancer in some animals will probably approximate to that in man, although the forms of cancer to which different species are most liable may differ. Since the greater frequency of cancer coincides with the later years of life, the span of life of human beings has long been one of the obstacles preventing a final

settlement of the question whether there is or is not a family liability to the disease. This obstacle is inoperative in the case of the mouse, with a short life of two to three years. Statements have been made of the greater frequency of cancer in the mice housed in certain cages, but no account has been taken of the total population of the cages for the period during which the tumours were observed, nor of the sex and age-constitution of the mouse population. In short, the data necessary to justify statements of relative frequency have not been ascertained. To baldly assert that epidemics occur, or that the disease must have been conveyed from one mouse to another because so and so many cases of cancer have occurred in one cage, as has been done in the most positive manner by the New York State Laboratory, Buffalo, U.S.A., is to ignore the rudiments of statistics. By breeding and in-breeding mice of cancerous stock we hope to finally settle the importance attaching to the apparent frequency of cancer in some strains of mice as compared with others. We have long had such observations in progress; but, since statistical studies on the incidence of cancer in animals require to be conducted with all the precautions accurate statisticians employ in dealing with the incidence of cancer in a human population, we are not yet able to institute comparisons between different communities of mice. These observations do not lend support to assertions of infection, any more than do our studies on the nature of experimental transference, and the means whereby immunity or protection may be conferred. The universality of the disease in man and animals, the biological law of its age incidence, the unique character of the proliferation and its continuation on transplantation, the peculiar nature of the measures which protect mice, as well as the intimate relations between normal and cancerous tissue, and the increased digestive activity induced to compensate for the building up of protoplasm in the tumours borne by otherwise normal animals, all point to the probability that cancer arises *de novo* in the individual attacked. This statement may seem to be mere dogmatic reiteration of an oft-expressed hypothesis; but experiment has removed this conception of the relation of cancer to the individual from the realms of hypothesis to the level of a well-considered theory, harmonising many isolated facts, and fruitful as the basis of further inquiry.

Finally I may point out, that if the comparative and experi-

mental investigation of cancer has revealed the fallacious nature of all reputed analogies with known forms of infection, the results obtained are essentially of constructive value. The cell problems being attacked now were unapproachable five years ago, as were also the relations existing between tumours and the organisms bearing them. Having recognised fully, and insisted from the outset of our investigations, that the reproduction of the lesions of cancer in normal animals by transplantation differed from the development of the disease *ab initio* in animals spontaneously affected, I have confined myself largely to describing the growth of cancer in the soil provided by normal animals, and to drawing some inferences as to the conditions obtaining in the development of cancer. I have shown that even in normal animals the soil plays an important part, and a part which can be modified at will. I have indicated that the soil may play an important part in the spontaneous appearance of cancer in individuals. I have also shown that the primary properties of cancer cells differ. Therefore the circumstances associated with the development of cancer require to be considered with reference to the two sets of factors last mentioned.

The extent to which the experimental method has already deepened our knowledge of the properties of cancer cells and of their relations to normal animals renders it probable, that its further application will throw light on the conditions underlying the association of the inception of cancer with the senescence of tissues, and on the increasing frequency of the disease as age advances, throughout the vertebrate kingdom.

THE USE OF THE ELECTROMAGNET IN PETROGRAPHY

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1. Introduction

It is well known that minerals exhibit varying degrees of permeability in a magnetic field, in consequence of which grains of one mineral can often be separated from those of others. In recent years the method of magnetic separation has become very important in the mining world. It is quite indispensable in a large number of ore-dressing operations, in which domain its utility is fully recognised.

In the laboratory also, this method has often been used with great advantage, more especially in the isolation of minerals for chemical analysis. Indeed, the magnet, and especially the electromagnet, must be regarded as a very useful piece of petrographical apparatus. Experience has shown that it is capable of extensive use qualitatively, while in special cases it yields quantitative results which are fairly accurate, particularly in the examination of sands.

Considering the ready and extensive applicability of this method in rock studies, there is scope for surprise that it has not been more widely adopted, for there can be little doubt that, among geologists, the practical utility of the method is much undervalued where not completely ignored. This may be due in some measure to unfamiliarity with the results already achieved. Hence it is proposed in this article to give an

account of what has been accomplished by the many workers who have used the magnetic method of examination in mineral and rock studies, and to call attention to certain meritorious work, especially by Delesse, which seems to have been unduly neglected by writers of text-books. The account will serve to emphasise the utility of the electromagnet in the examination of grains and fragments of rock-forming minerals.

2. Historical Review

During the latter half of the eighteenth century, numerous observations were made by various workers, including Romé de Lisle, Delarbre, and Haüy, on the magnetic polarity exhibited by crystals of magnetite and hæmatite.

Among geologists, H. B. de Saussure early recognised the value of the magnet, and included it in his list of instruments needful to a field geologist. In vol. i. of his *Voyages dans les Alpes*, published in 1779, he discussed, in a most elaborate and interesting manner, the attractive effect of garnets on a magnetic needle, and described a delicate means for observing this effect; Saussure even proposed to use this property as a means of estimating the amount of iron in a garnet. He also dealt with the magnetic influence of mountains as observed by a magnetometer. As an explanation of this influence he put forth the "rock-magnetism" theory, and considered that the cumulative magnetic effect of the ferriferous minerals (hornblende, etc.) of which the mountains were composed, was quite sufficient to account for the effects observed. Further, he indicated the value of the magnetometer as a means of locating bodies of iron ore. ("Cette observation prouve la sensibilité du magnétometre (1) l'usage que l'on pourroit en faire pour decouvrir des mines d'aimant ou de fer": *loc. cit.* tome iv.).

Incidentally, and as an illustration of the way in which modern authors are apt to overlook the credit due to early workers, it may be pointed out that Haanel[1] attributes the discovery of this method of locating iron ores to Wrede (1843). Again, with respect to Saussure's observations on the magnetic disturbance exercised by mountains, as evidenced in the region of the Alps,

[1] *On the Location and Examination of Magnetic Ore Deposits by Magnetometric Measurements*, Department of the Interior, Ottawa, Canada, 1904.

we may note that M. de Lapparent [1] is content to attribute early observations on this matter to Kreil (1849), who also worked among the Alps, and to Locke (1846), who made investigations in North America.

Korda [2] states that as early as 1778 Brugman, of Leyden, had described many minerals as being magnetic, including those which contained iron and cobalt. The same author states that in 1792 Fullerton obtained in England a patent for separating magnetic from non-magnetic materials by means of permanent magnets.

The geologists of this period were familiar with the wide distribution of magnetite in eruptive rocks; they were in the habit of extracting it from crushed rocks by means of a magnet, and of noticing that the rocks which contained it affected the magnetic needle. In 1794 Dolomieu [3] characterised his group of compact basic volcanic rocks as having, among other things, a marked effect on the magnetic needle. He observed that specimens of certain lavas exhibited a well-marked polarity, which was retained by the fragments when the specimen was broken.

In 1800 Bellevue [4] recommended the partial crushing (demi-trituration) of rocks, and mechanical analysis (*analyse mécanique*) of the fragments, including elutriation and treatment with a bar magnet. In this way he made a thorough examination of the leucite-nepheline-melilite-basalt of Capo-di-Bove, making a fairly full record of its mineral composition. He concluded his interesting memoir by stating his belief, that the adoption of the methods he describes would be a potent means of discovering new minerals, and of elucidating the nature of rocks. It is instructive to note the acute observations made by these early workers, and not a little surprising to find that they achieved so much with the limited means at their disposal. Bellevue especially emphasised the importance of examining the sands which had resulted from the disintegration of rock masses.

As if to show that he had appreciated the importance of

[1] *Traité de géologie*, 1906, tome i. p. 106.

[2] *La séparation électromagnétique et électrostatique des minerals*, Paris, 1905.

[3] "Suite de mémoire pour servir d'explication à la distribution méthodique de tous les produits volcaniques," *Journal de Physique*, etc., 1794.

[4] "Mémoire sur les cristaux microscopiques," *Journal de Physique*, tome li. 1800.

Bellevue's hint, Cordier[1] proceeded to examine a large number of sands obtained from the river beds, lake and sea shores, in the vicinity of volcanoes. By means of a bar magnet he separated the magnetite from these sands, and proved it by analysis to be richly titaniferous. Throughout his later work on the volcanic rocks themselves, Cordier used the magnet freely in his researches, and was able to prove the world-wide distribution of titaniferous magnetite as a constituent of eruptive rocks.[2]

The introduction by Sturgeon in 1825 of an electromagnet bent in horseshoe fashion, disclosed the fact that by such means a magnetic field of great intensity could be obtained. Following this method of construction, M. Pouillet, about 1831, had an electromagnet made which was capable of supporting a weight of about 800 kilograms. With it he was able to prove that many substances were attractable by a magnet which were not previously known to be magnetic.

About 1845 Faraday[3] made his famous experiments with the electromagnet, and examined a large number of substances, including some minerals, distinguishing them as paramagnetic or diamagnetic according as they were attracted or repelled by the poles of a magnet.

It is worthy of note that Faraday's paramagnetic substances included manganese, chromium, cerium, and their compounds. The magnet used by Faraday is interesting because of the fact that he used adjustable pole pieces, which enabled him to vary the distance between the poles, and thus to vary the intensity of the magnetic field. ("The poles are 6 in. apart, the ends are planed true, and against these move two short bars of soft iron 7 in. long, $2\frac{1}{2}$ in. wide, and 1 in. thick, which can be adjusted by screws and held at any distance less than 6 in. from each other. The ends of these bars form the opposite poles of contrary name; the magnetic field between them can be made of greater or smaller extent, and the intensity of the lines of magnetic force be proportionately varied": *loc. cit.* vol. iii. p. 29.) Such adjustable pole pieces are very useful as a means of varying the intensity of the magnetic field, in making mineral separations.

[1] "Recherches sur différens produits volcaniques," *Journal des Mines*, No. 124, vol. xxi. 1807.

[2] "De la manière d'être du fer titané dans les roches volcaniques, et du rôle qu'y joue ce minéral," *Journal des Mines*, No. 133, vol. xxiii. 1808.

[3] "Experimental Researches in Electricity," London, 1855.

They require, however, to be somewhat modified, as will be pointed out later.

Contemporary with Faraday, Pluecker[1] examined some minerals and many other substances by means of the electromagnet. He expressed the relative permeability of these substances by a series of numbers, taking iron as a standard of comparison, in somewhat the same manner as Delesse (see below).

While Faraday, Pluecker, and others were directing their attention to the purely physical aspect of magnetic properties, two French geologists, Fournet and Delesse, attended to the matter more particularly with reference to its bearing on minerals and rocks. Fournet's work[2] was directed towards an explanation of the anomalies of terrestrial magnetism. For this purpose he studied the magnetic properties of all the important rock-forming minerals, not overlooking the fact that even the felspars, due to ferromagnetic impurity, often affect the magnetic needle. He distinguished magnetite, hæmatite, and ilmenite as being magnetipolar. Other magnetic minerals he described as showing simple magnetism—*i.e.* they were attracted by a magnet, but gave no evidence of polarity. He discussed the varying magnetic characters exhibited by rocks of different types as well as minerals, and upon this basis proceeded to deal with the general effects of different rock masses (granites, basalts, limestones, etc.) in modifying the earth's magnetic field. It is noteworthy that Fournet did not use an electromagnet in his observations. He relied on the use of a simple permanent magnet, following the methods of H. B. de Saussure and Haüy. Fournet's paper is a compendium of careful observations, and strikes one as being a document of somewhat considerable value. Unfortunately, it appears not to have received the attention it deserves. As an amplification of the earlier work by Saussure, it merits careful consideration by those who are interested in the bearing of petrography on the subject of terrestrial magnetism.

Of all the contributions dealing with the application of the electromagnet to the study of minerals and rocks, those made

[1] *Poggendorf's Annalen der Physik.*, Band lxxiv. p. 321, 1848.

[2] "Aperçus sur le magnétisme des mineraux et des roches, et sur les causes de quelques anomalies du magnétisme terrestre," *Annales des Sciences, Société d'Agriculture, etc., de Lyon*, tome xi. 1848.

by Delesse rank among the most important.[1] It is all the more necessary to emphasise this work by Delesse since it appears to have been hitherto almost completely ignored. He regarded the magnetic power (*pouvoir magnétique*) of minerals as being to some extent a character of specific importance, and made a magnetically quantitative comparison of a large number of minerals. To do this he crushed them, and experimented with grains of uniform size in a field of constant intensity. The maximum weight of such grains adhering to the poles of the magnet, under uniform conditions, was taken as an index of what he called the magnetic power of a mineral. Thus, two minerals had the same magnetic power if, under similar conditions, equal weights of them adhered to the poles of the magnet. If the adhering weight of the grains of one mineral was twice that of another, the magnetic power of the former was twice that of the latter. In short, the magnetic power was proportional to the weight of the adhering grains, and consequently

$$x = \frac{p}{P}$$

where x = magnetic power of a mineral,
p = maximum weight of adhering grains of this mineral,
P = maximum weight of adhering grains of some standard substance, the magnetic power of which may be taken as unity.

This method of comparison has been stigmatised as primitive and objectionable; but although it cannot lay claim to great exactitude, it is at least simple and feasible, and proves to be a very efficient and useful means of comparison. Especially when we consider the fact that the magnetic field used is not uniform, and that the permeability of a mineral is generally far from constant, it seems clearly undesirable to sacrifice practical utility, in the effort to attain a more than requisite degree of accuracy.

Using this mode of comparison, Delesse examined numerous minerals, taking steel as his standard. The following list is compiled from his results; the number opposite a mineral

[1] See particularly "Sur le pouvoir magnétique des mineraux et des roches," *Annales des Mines*, 1848, tome xiv. p. 429; also abstract of the same in *Annale de Chimie et de Physique*, tome xxvi. 1849.

represents its magnetic power, assuming that of steel to be 100,000:

Magnetite	64,121	Pleonaste	73
Titaniferous magnetite	48,405	Pistacite	49
Ilmenite	5,764	Allanite	47
Pyrrhotite	4,718	Jasper	33
Hæmatite	2,352	Biotite	20
Hypersthene	687	Idocrase	18
Augite	559	Axinite	17
Garnet (hyacinth)	294	Sahlite	14
Olivine	250	Kyanite	12
Hornblende	237	Talc	12
Chromite	136	Aventurine	7
Siderite	120	Andesine	5
Piedmontite	80	Tourmaline (black)	4
Staurolite	77	Oligoclase (sunstone)	4

The above list is a very incomplete representation of Delesse's results. In many cases he compared the different varieties of a mineral, and the results exhibited considerable variation due to differences in composition and the amount of impurity. For this reason, the numbers given in the above list cannot be regarded as being exact in their significance, especially when they are considered apart from the composition and physical condition of the minerals concerned; but they serve to illustrate, in a general way, the variable magnetic permeability among minerals.

Delesse exercised caution in making his comparative measurements, and avoided errors due to variation in current, etc. The inconstancy of the current generated by his battery led him to suggest that it was preferable to use a strong permanent magnet in making observations on the comparative magnetic powers of minerals. He pointed out that a compound horseshoe magnet was quite powerful enough for the purpose, while it had also the great advantage of being constant.

His comments on the peculiarities exhibited by the different groups of minerals are very interesting. These comments were based largely on the view that the chemical constituents of a mineral asserted their separate magnetic effects, even in a state of combination. Thus he observed that the presence of iron, manganese, cerium, and other paramagnetic elements tends to increase the magnetic power of a mineral, while the presence of silica, alumina, lime, water, fluorine, and other diamagnetic constituents tends to diminish it. A broad survey of minerals

lends much support to this view, and it explains many peculiarities somewhat consistently. But it has long been known that the influence of any chemical constituent in determining the magnetic power of a substance is not strictly additive, and is seriously modified by its mode of combination. Thus compounds of copper with the halogens are said to be paramagnetic,[1] although pure copper and the halogens are separately diamagnetic. Alloys exhibit some striking effects: for example, iron and nickel, both of which are powerfully magnetic, are stated to be capable of yielding an alloy which is practically non-magnetic. Such examples, however, appear to be somewhat exceptional. The investigations of St. Meyer[2] show that, as a rule, a compound consisting of two diamagnetic elements is itself diamagnetic, and that a compound consisting of two paramagnetic elements is itself paramagnetic. But the rule does not apply additively in a quantitative sense: for instance, the magnetic power of a compound consisting of two paramagnetic elements is less than the sum of the separate magnetic powers of the two elements. Again, when water enters into the composition of a mineral, it does not exert its full diamagnetic effect. The physical condition of a mineral is also an important factor in determining its magnetic permeability.

Some of Delesse's observations on the magnetic characteristics of the various groups of minerals are worthy of being recounted. The members of the amphibole group showed variation in accordance with the iron percentage. Actinolite exhibited a feebler power than common hornblende; anthophyllite was feebler still, while tremolite was practically non-magnetic. The pyroxene group showed a variation analogous to that of the amphiboles.

He thought the relatively feeble power displayed by biotite might be explained by the presence of fluorine, which he stated to be probably diamagnetic. Pluecker had already shown that the other halogens were diamagnetic. He was surprised at the feeble magnetic power displayed by the chlorites, seeing that they were comparatively rich in iron oxides and poor in silica. As, however, he included water in his list of diamagnetic substances, we might have expected him to attribute

[1] St. Meyer states that this behaviour is due to impurity.

[2] "Magnetisirungszahlen anorganischen Verbindungen," Stefan Meyer, *Wied. Ann. der Physik*, Band lxix. 1899.

the low magnetic power of the chlorites to their state of hydration, containing as they do about 12 per cent. of water. In this connection we may note that the contrast between hæmatite[1] and limonite is a good example of the effect of hydration in diminishing the magnetic power of a mineral. Another example of this is copper sulphate, which is distinctly more magnetic in the anhydrous than it is in the hydrated condition.

Delesse attributed the low magnetic power of black tourmaline to the diamagnetic effect of boron. He observed that the magnetic power of sulphides diminished very rapidly as the percentage of sulphur increased: thus, pyrrhotite (Fe_nS), with rather less than 40 per cent. of sulphur, was highly magnetic, while ordinary pyrite (FeS_2), with about 54 per cent. of sulphur, was practically non-magnetic. But here, again, we are tempted to remember that sulphur is a diamagnetic element.

Before quitting this consideration of Delesse's work, it is important to remark that he crushed certain French biotite-granites, and, by the electromagnetic method, not only isolated the biotite, but estimated quantitatively its proportion to the bulk of the rock. ("Le mica est faiblement magnétique, cependant les micas noirs-brunâtres le sont assez pour adhérer facilement à l'électro-aimant. Je me suis même servi de cette propriété pour déterminer la proportion de mica qui se trouve dans quelques granites de la Normandie et de la Bretagne qui sont employés au pavé de Paris."[2])

This appears to be the first record of the use of the electromagnet in the isolation of feebly magnetic minerals from rocks, and the quantitative estimation of the same. But the method is evidently only a logical extension of that adopted by the earlier workers, Bellevue and Cordier, for the separation of magnetite.

In 1872 Fouqué published in the *Comptes rendus* (tome lxxv.) an abstract of a memoir entitled "Nouveaux procédés d'analyse médiate des roches et leur application aux laves de la dernière eruption de Santorin."[3]

[1] Excluding those highly magnetic crystals which contain ferrous oxide, probably due to intergrowths of magnetite.

[2] *Annales des Mines*, 1848, tome xiv. p. 479.

[3] For the full memoir see *Mém. prés. par divers savants à l'Académie des Sciences*, tome xxii. No. 11, 1876.

In this memoir Fouqué described what he called a new mechanical process, by means of which he had been able to isolate the felspars of the Santorin lavas. In this process the rock was crushed and the crushings submitted to the action of a strong electromagnet. The iron ores, the ferromagnesian minerals, and the partially differentiated ground of the rock were extracted, leaving a non-magnetic residue of felspar. The apparatus used by Fouqué consisted of an electromagnet put into action by a Bunsen battery, and was essentially similar to the apparatus used by Pouillet, Delesse, and others. Fouqué also appears to have used a dynamo in some cases, as a substitute for a Bunsen battery. His results are very interesting, and prove convincingly the importance of the electromagnet as a means of isolating minerals. By its aid, and in conjunction with other methods, he was able to demonstrate that a given specimen of lava might contain quite a medley of plagioclases. He also proved that two varieties of pyroxene were present, both rich in lime, but differing considerably in the proportions of magnesia and ferrous oxide; also two olivines which differed in the amount of ferrous oxide present.

It is quite clear, however, from the description which has been given of the work done by Delesse and others, that Fouqué, using the electromagnet in his researches, was using an old and not a new process. It seems scarcely credible that Fouqué could have been unaware of the work already done in this field by his fellow-countryman Delesse, and published in a journal so well known as the *Annales des Mines*. Even in the *Minéralogie micrographique* of 1879, in which Delesse's other work is fully recognised, Fouqué's error remained unrepaired, and it has unfortunately been persisted in by later text-book writers.

Doelter,[1] following Fouqué, and apparently unaware of any important results other than those which the latter obtained, applied the electromagnet to the study of rocks and minerals. He wrongly attributes to Fouqué the priority for using an electromagnet in the systematic study of weakly magnetic rock-forming minerals. He makes matters worse by referring

[1] "Ueber die Einwirkung des Electromagneten auf verschieden Mineralien und seine Anwendung behufs mechanischer Trennung derselben," *Sitzungberichte*, Wien, 1882, Band lxxxv. Abteilung i.

us to the already quoted abstract by Delesse in the *Annales de chimie et de physique*, and misrepresents it by telling us that Delesse had only been able to separate magnetite from his rocks. ("Eine ähnliche Methode wandte Delesse zu Bestimmung des Magnetismus von Gesteinen und Mineralien an. Er konnte jedoch dadurch nur den Gehalt von Magneteisen bestimmen, denn dieses war es, welches bei seinen Versuchen entrahirt wurde.")

Like Fouqué, Doelter operated with magnetic fields of different intensities, obtained by varying the number of the Bunsen cells (2 to 8) which he used. In this way he examined many minerals, and gave a list showing the amounts by weight of the crushed minerals attracted to the poles of the magnet from approximately 0·25 gram in each case, varying the strength of the field according to the ease with which the mineral was attracted. This list in some measure resembles that prepared earlier by Delesse, but is much less complete. Further, for comparative purposes, Delesse's method of using a field of constant intensity and a standard for comparison is decidedly preferable to the method adopted by Doelter.

Another, though perhaps less effective method of comparison used by Doelter, was to operate with a field of constant intensity, and to determine the maximum distance from the poles at which grains of uniform size were lifted by the magnet. The results thus obtained are instructive, and are as follows:

	Millimetres		Millimetres
Magnetite	12·0	Augite (Santiago basalt)	3·0
Hæmatite	6·0	Hornblende	2·8
Siderite	5·0	Pleonaste	3·7
Lievrite	4·7	Augite (Vesuvius)	2·5
Almandine	4·9	Idocrase	2·4
Hedenbergite	4·3	Tourmaline	2·1
Augite (Pico da Cruz)	4·0	Actinolite	1·7
Augite (Praya)	3·7	Olivine	0·8
Epidote	3·7	Vivianite	0·6

Doelter also gives another list, as follows, in which those minerals under the same number have nearly the same magnetic value, starting with the most magnetic and ending with the least:

1. Magnetite.
2. Hæmatite, ilmenite.
3. Chromite, siderite, almandine.

4. Lievrite, hedenbergite, limonite.
5. Augite (15 to 20 per cent. of iron oxides), pleonaste, arfvedsonite.
6. Hornblende, augite (poor in iron), epidote, pyrope.
7. Tourmaline, bronzite, idocrase.
8. Staurolite, actinolite.
9. Olivine, pyrite, vivianite.
10. Biotite, chlorite, rutile.
11. Haüyne, diopside, muscovite.
12. Nepheline, leucite, dolomite.

It has been already mentioned that, owing to variations in composition exhibited by many minerals (especially in point of iron percentage), such lists ought not to be regarded as being accurate.

Perhaps the most noteworthy feature of Doelter's work with the electromagnet, was that of his experiments on crushed rocks. He attempted the separation of the mineral constituents as far as possible by the electromagnet alone, and stated the results quantitatively. But the fact that he operated on the dry crushings detracts seriously from the merit of the results he obtained. The character and extent of his separations may be seen from the following instances compiled from his results.

Nepheline Syenite, Antao Island

Magnetite	5 per cent.
Augite and hornblende	30 „
Nepheline and felspar	65 „

Phonolite of Praya

Magnetite	4 per cent.
Augite	11 „
Felspar and nepheline	85 „

Compact Basalt, Santiago Island

Magnetite	9 per cent.
Augite	39—46 „
Olivine	6—10 „
Plagioclase	32—44 „

Of the above results, we may take that for the nepheline syenite as possibly accurate. In the cases of the phonolite and basalt, however, we may reasonably inquire what has become of the undifferentiated ground of the rock. Indeed, it is fairly obvious that accurate results cannot be got by physical methods when dealing with hemicrystalline or very

compact types of rocks. The method can only be trusted quantitatively when applied to holocrystalline rocks, in which the particles obtained by crushing consist of individual (*i.e.* unmixed) minerals. In fairness to Doelter, however, it should be admitted that he was aware of the fact that in the case of hemicrystalline rocks the results were somewhat unsatisfactory. But in his enthusiasm for the method he went so far as to claim even for these, that they were of value as controlling and supplementing the results of chemical analysis. This claim may be fully respected when applied to holocrystalline rocks, but its assertion in the case of hemicrystallines is by no means warrantable.

The conclusions to which Doelter was led by the results of his experiments with the electromagnet summarise in an admirable way the scope of that instrument's utility as applied to rocks. His conclusions were: (1) that the electromagnet is of great service in petrographical studies; (2) that minerals can often be isolated by its aid which cannot be conveniently isolated by any other means; (3) that it is especially useful when applied in conjunction with the use of heavy liquids; (4) that the separations made by it, even when incomplete, are generally of great use, while in some cases they may be taken as being of somewhat definite quantitative value.

In a criticism of Doelter's work, Pebal[1] called attention to the fact that, when operating on fine crushings, the separation can be made more efficient by keeping the crushed fragments immersed in water during the process. Doelter attempted to refute this recommendation made by Pebal; but a comparison of experimental results indicates that the recommendation is a very good one, and that its adoption substantially increases the accuracy of the results for the finer crushings.

Mann[2] used the electromagnet to isolate augite for analysis from certain phonolites. He followed Pebal in the use of the wet method of separation, and denounced the dry method of Fouqué and Doelter. Mann also adopted a novel procedure in making some of his separations. He placed his magnet horizontally, and allowed the mineral fragments to fall in water

[1] *Sitzungberichte*, Wien, 1882, Band lxxxv. Abteilung i.

[2] "Untersuchungen über die chemische Zusammensetzung einiger Augit aus Phonolithen und verwandten Gesteinen," *Neues Jahrbuch für Mineralogie*, etc., 1884, Band ii.

through a glass tube, which was placed vertically between the poles of the magnet. The tips of the poles were only 5 mm. apart, and were chisel-edged, so as to concentrate the lines of force. Mann had little or no confidence in the use of the electromagnet quantitatively. He made numerous attempts to estimate the amount of augite in his phonolites by this method, doubtless encouraged by Doelter's example, but his results showed so much variation that he abandoned them as untrustworthy. As already pointed out, however, such rocks can scarcely be expected to yield even approximately accurate quantitative results.

It is beyond the scope of this article to deal with the many and elaborate inventions made during the last twenty years or so, by Wetherill and others, in applying the electromagnet on a large scale to ore-dressing operations. Those requiring information on this matter may be referred to the book by M. Korda already quoted (published by L'Éclairage Électrique, 40, Rue des Écoles, Paris), and also to the descriptions given by Richards,[1] Langguth,[2] and Witter.[3]

From the foregoing review of work done on the subject of the magnetic method of mineral separation, it is clear that the possibilities of the method have been somewhat fully considered by the various workers who have used it. In spite of this fact, however, any one wishing to use the electromagnet in petrographical work would find some difficulty in getting helpful guidance as to the best mode of procedure. To such, the following practical hints, tested in the course of some experience with the electromagnet, may be found useful.

3. The Apparatus Required

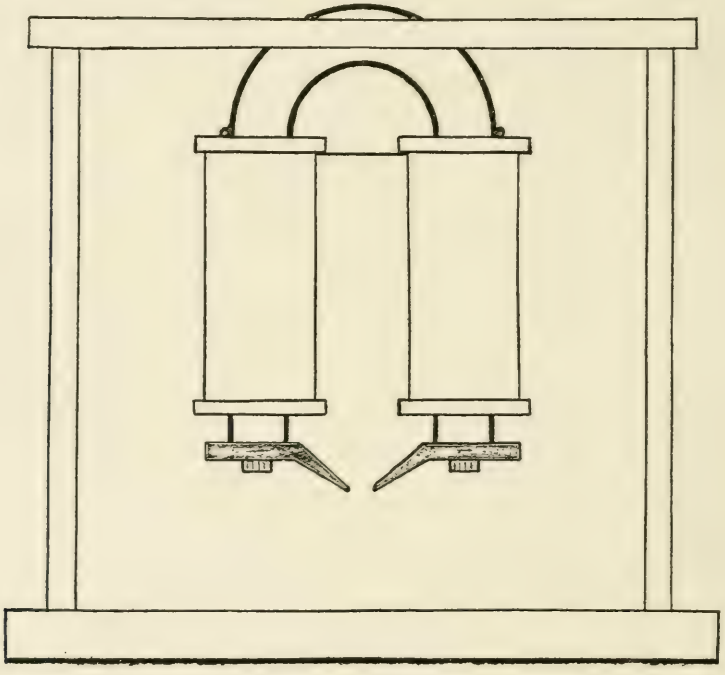
If expense can be disregarded, a magnetic separator of the Wetherill pattern is well worth having, especially when experiments are to be done on a large scale. A good example of the use of such a separator in this way is provided by a recent investigation as to the commercial value of certain heavy placer

[1] *Ore Dressing*, vol. ii. 1903. Published by *The Engineering and Mining Journal*.

[2] "Die Entwicklung der magnetischen Aufbereitung," *Zeitschrift f. Electrochemie*, Band vi. 1899.

[3] *Zeitschrift f. Angewandte Chemie*, 1899.

sands in the United States.[1] Such an instrument, however, is a decided luxury, and goes far beyond the needs of the average laboratory investigation. For ordinary purposes it suffices to use a magnet such as that illustrated by the accompanying figure. This electromagnet is one of the ordinary pattern, and is supported on a wooden frame. It consists of a cylindrical piece of soft iron, 1 in. in diameter, bent into U-shape, and having vertical limbs about 6 in. long and 3 in. apart. Each limb



is provided with a bobbin, on which is wound seven layers of insulated wire (16-gauge), each layer having about forty turns. Two pole pieces, consisting of soft iron about $1\frac{1}{4}$ in. wide and $\frac{1}{3}$ in. thick, are slotted so as to move over screw clamps which fit into the ends of the limbs. By this means the pole pieces can be adjusted in any desired position. As already pointed out, this was the device adopted by Faraday to secure a magnetic field of strong but variable intensity.

[1] *Investigation of Black Sands from Placer Mines*, by Day and Richards. Bulletin No. 285, p. 150, United States Geological Survey, 1906.

The adjacent portions of the pole pieces should be bent downwards, but not at right angles, narrowing slightly and thinning towards the tips, somewhat after the manner suggested by Doelter. The practice of having the adjacent ends of the pole pieces bent downwards at right angles in the way recommended by Rosenbusch[1] is very faulty. It is clear that with such an arrangement the lines of force must be distributed over all the space between the vertical plates, and as only the tips are used, most of the magnetic force is thereby wasted.

The magnet may be conveniently put into action by an eight-volt secondary battery of the portable Lithanode type. It is an additional convenience if, as is usually the case, the battery be arranged so that two, four, six, or eight volts can be obtained, as this affords an additional means of varying the intensity of the magnetic field.

Instead of a secondary battery an ordinary current, such as is used for lighting purposes, may be used, in combination with some form of resistance apparatus. A dynamo or a Bunsen battery may also be used as a substitute.

The wire connecting the battery with the electromagnet should be provided with a simple lever-switch arrangement, by which the current can be switched on or off as required.

The simple apparatus thus outlined will do all that is necessary in the way of isolation and separation, with such small amounts as one usually uses in the examination of sands and rocks generally.[2]

It may be cheaper, but not so convenient, to use a compound and permanent horseshoe magnet, as suggested by Delesse. Moreover, such a permanent magnet may be provided with adjustable pole pieces, like those already described for the electromagnet. But, in the absence of some mechanical device for suspending the action of the magnet on the grains, and thus permitting them to fall, such an arrangement is scarcely suitable for making separations.

[1] See *Mikroskopische Physiographie der petrographisch wichtigen Mineralien*, vol. i. part i. p. 431, 1904.

[2] This apparatus, including an eight-volt secondary battery, an electromagnet, and a switch, is supplied for the modest sum of about £3 10*s*. by the General Electric Co., 71, Queen Victoria Street, E.C. The electromagnet alone costs about £1 10*s*.

4. Preparation of Material

In the case of sands, the separation can usually be made without crushing, as the grains are generally small and of a simple character; but it is often convenient to sift, in order, as far as it is practicable, to secure uniformity of size among the grains.

With rocks, the degree of crushing required depends upon the texture, but as a rule the diameter of the grains obtained should not exceed 0·25 mm. The crushing should be done so as to secure a minimum of powder, especially in quantitative operations. This is effected by sifting at frequent intervals during the crushing process. The powder should be washed off, and, if necessary, treated separately in the wet way according to Pebal's suggestion. In any case the treatment of the powder usually gives unsatisfactory results, although for quantitative purposes it obviously cannot be neglected. On this point it is important to note that some minerals powder more readily than others, hence the proportion of any mineral present in the powdered portion will differ from that of the same mineral in the coarser portion of the crushings.

For the purposes of isolation, or in merely qualitative separation, the powder may be washed off and rejected after a cursory examination with the microscope.

5. Separation of the Minerals

Magnetite, if present, should first be extracted by a weak permanent magnet—an operation which is facilitated by covering the poles with a movable paper cap. The portion thus extracted should be carefully examined, as it may contain pyrrhotite and even hæmatite, both of which are very strongly magnetic. Ilmenite is not extracted by a weak magnet, except in the finest powder, and then only to a small extent.

The remainder of the grains are placed on cardboard, and, assuming the current to be switched on, are presented to the poles of the electromagnet. The cardboard is then removed, the current shut off, and the adhering grains allowed to drop into a cardboard box placed under the poles. This is continued till no more grains adhere to the poles. A certain amount of non-

magnetic and less magnetic material is, as a rule, mechanically enclosed in the most magnetic portion of the first separation. Hence the operation should be repeated till no residue remains behind, or till the separation is satisfactorily made. In the presence of fine powder it is practically impossible to get a good separation in the dry way, due to the fact that non-magnetic grains are mechanically enclosed in a mesh of magnetic grains—a defect which seems to be largely eliminated by having the grains immersed in water during the separation.

The actual details of the separation will depend upon the character of the specimen, and must be left to the judgment of the operator. A little experience suffices to indicate the best course to adopt in any given case. If a number of minerals of suitably varying permeability be present, the most permeable are first of all separated in a comparatively weak field, after which the field is intensified to secure the separation of a less magnetic ingredient.

The manner in which the grains are presented to the poles depends upon whether they are strongly or weakly attracted. In the former case the grains should not be permitted to touch the poles, while in the latter case the poles and the grains should be brought into contact. Thus, if the poles be inserted in a mixture of ilmenite and garnet grains, with a field of medium intensity, both minerals are extracted. But if the grains be not allowed to touch the poles, it will be found that, at a certain distance, the ilmenite is withdrawn and the garnet left behind. Further, by this means less non-magnetic material is enclosed with the ilmenite than is the case when the poles are inserted among the grains. On the other hand, in the case of a mixture of monazite, zircon, and quartz, the poles may be inserted among the grains, in order to extract the monazite, which is only slightly magnetic.

It is, of course, not possible by the magnetic method to separate minerals which agree or differ only slightly in permeability. For instance, hornblende cannot be separated from augite of similar composition ; garnet and rhombic pyroxene are commonly inseparable ; and some chromite cannot be separated from garnet.

As an example of separation, we may cite the following. The specimen was a concentrate obtained by washing a river sand. The minerals present were ilmenite, magnetite, garnet,

zircon, rutile, monazite, sillimanite, and quartz. The result of the magnetic separation was as follows:

	Per cent.
Magnetite	about 1·3.
Ilmenite, Garnet	„ 70·5 (only a trace of garnet).
Monazite	„ 5·6.
Rutile, Zircon, Sillimanite, Quartz	„ 22·5 (chiefly zircon).

In this case the monazite was almost completely isolated from the other ingredients, and the chief object of the separation was to determine its amount quantitatively, so as to get an approximate estimate for the amount of thoria in the total concentrate. In such an instance, when once the amount of thoria peculiar to the monazite grains of a district has been determined by chemical analysis, it becomes an easy matter to estimate the approximate thoria percentage of a concentrate by magnetic separation alone. Such estimates usually agree fairly closely with the results of chemical analysis.

Even in the absence of exact quantitative results for each mineral, the separation of the sand into groups of constituents gives a very clear idea of its mineral composition, and greatly facilitates examination with the microscope.

Another example of the usefulness of the electromagnet, especially when used in conjunction with other methods, may be taken from its application to a crushed rock. It is an especially useful means of isolating, or observing the presence of, certain accessory minerals in small quantities, such as apatite, rutile, zircon, and pyrite. The rock in question was an olivine gabbro; it was partially ophitic and medium-grained in texture. Twenty grams of the crushings were treated with the hand magnet to extract the magnetite. The remainder was treated with the electromagnet in a fairly strong field and divided into two parts: the magnetic portion was found, on examination with the microscope, to consist of a mixture of pyroxene (partly rhombic), olivine, hornblende, and brown biotite; the non-magnetic portion consisted almost entirely of plagioclase, with a little apatite and zircon, together with traces of pyrite and quartz. The separation

was somewhat imperfect, and the following result can only be regarded as approximately correct :

	per cent.		per cent.
Magnetite (titaniferous)	2·9	Plagioclases	
Pyroxene		Apatite	
Brown biotite		Zircon	
Green hornblende	43·0	Pyrite } traces	54·0
Olivine		Quartz }	

The non-magnetic group, which, as already stated, consisted almost entirely of plagioclases, was then treated with heavy liquids. In this way the plagioclases and apatite were each isolated. From the zircon and pyrite thus remaining, the zircon was isolated by heating till the pyrite was partly oxidised, after which it was separated magnetically. The zircon thus isolated consisted of crystals of the ordinary type, colourless, and having the characteristic inclusions. In amount they were almost too small to be weighable, forming less than 0·01 per cent. of the rock, and yet they proved to be substantial objects for microscopical study.

Some idea of the effectiveness of this method of dealing with small quantities of zircon may be gathered from the following considerations. The largest zircon crystal obtained had dimensions about 0·5 × 0·1 × 0·1 mm. Ten such crystals weigh less than 0·25 mg., though they can be handled with ease for microscopical purposes. Such an amount of zircon obtained from 20 grams of the rock would amount to about 0·001 per cent.

The above process for determining the presence of small quantities of zircon is to be recommended, as it is a simple one and takes little time. Moreover, zircon in such small quantities appears to be frequently present even in the most basic rocks, such as olivine gabbros and basalts, and it is then practically impossible even to detect its presence by chemical methods. Further, it would appear that zircon in such small quantities generally eludes observation in thin sections, judging from the rarity of the cases in which it is mentioned in descriptions of basic igneous rocks. It should be recalled that Chrustschoff, partly by elutriation, and partly by treatment with acids, isolated small quantities of colourless zircon from many varieties of rocks. One of the rocks he examined was a basalt,

from 300 grams of which he obtained 0·022 gram of zircon—*i.e.* about 0·007 per cent. The method used by Chrustschoff,[1] however, lacks the neatness and precision which is attainable by the use of the electromagnet in combination with the use of heavy liquids.

6. Conclusion

To enter at further length on a description of the magnetic treatment of rock-forming minerals would unduly prolong this article. Sufficient has been stated to indicate that, to students of petrography, the electromagnet is an instrument of considerable value. It only remains to point out that, like every other means of observation, its sphere of usefulness is a limited one, and too much should not be expected from it. The presence of magnetic impurities in otherwise non-magnetic minerals is obviously a disturbing factor of great importance. For this reason, the use of the magnet should in all cases be controlled by examination with the microscope, and no separation should be taken as being exact, without confirming the purity of the separated portions in this way. Used in this manner, the method of magnetic separation is one which gives great assistance as a mode of physical rock analysis. Those who are anxious to make themselves in some measure independent of rock sections, and to avail themselves of the many advantages offered by a systematic study of crushed rocks, will find it a most useful adjunct in a somewhat neglected field of work.

[1] "Beitrag zur Kentniss der Zirkone in Gesteinen," *Tschermak's Mittheilungen*, Band vii. 1886.

THE COAGULATION OF THE BLOOD

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1. Introductory

In all vertebrate and invertebrate animals which possess either a true vascular or a pseudo-hæmal system, it is known that the liquid within these channels is in a state of continual or intermittent movement during life. In order that this condition shall be maintained, the blood or lymph or tissue-fluids contained within both the lymphatic and blood-vascular system must possess only a small degree of viscidity, which, apart from the suspended morphological bodies, depends upon their content in protein substances. The actual viscidity of blood varies scarcely at all during life, though its viscosity appears to vary within limits and to be largely dependent upon the corpuscular richness; an excessive number of corpuscles in the unit volume increases the friction-coefficient of the blood, thereby throwing extra work upon the heart. Blood which escapes from the blood-vessels into the extra-vascular regions of the body may or may not exhibit that change in viscidity which all

physiologists have recognised to be, next to the colour, its most distinctive feature outside the body. A gradual change in consistence takes place from the moment blood is shed, until, as a firm jelly, it forms a clot which is a cast of the vessel containing it. The plasma or fluid in which during life the red corpuscles and leucocytes floated has thus formed a single coherent mass of fibrin and corpuscles. This forthwith commences to shrink, with the consequent production of a spontaneously non-coagulable liquid or blood-serum.

In 1891 Lister[1] drew attention to the fact that, although blood may clot, no marked retraction of the clot due to a shrinking of the fibrin occurs when blood has been received in a vessel rendered sterile by heat or by washing it out with weak perchloride of mercury. A similar phenomenon is frequently seen when blood is collected for obtaining antitoxic sera. Hayem[2] and his pupils have also observed the absence of serum from the clotted blood of patients with pernicious anæmia or purpura hæmorrhagica. They attach a special importance to this non-retraction of the clot for the positive diagnosis of pernicious anæmia and most forms of purpura hæmorrhagica, and consider the phenomenon is of even greater value than a histological examination for the recognition of the former disease. This non-retraction of the clot is at present unexplained, but since it is a feature of clotted plasma in contrast to normal clotted blood, it is probably dependent in some way upon influences exerted on the fibrin by the imprisoned corpuscles or, according to Hayem, the blood-platelets, since the plasma of birds, which may contain only a few or no corpuscles, will, on being caused to clot by the addition of tissue-extract, show no subsequent retraction of the clot—a fact which has been noticed by Delezenne,[3] Fuld,[4] and Spangaro.[5] The fibrin, which forms but a very small amount of the bulk of the clot, subsequently alters in its physical characters, becoming more soluble. The extent of fibrinolysis and the rate at which this occurs differs in different animals; it is also a subject of much interest, since a study of the conditions under which it occurs

[1] *Lancet*, p. 1082, 1891.
[2] *La Semaine médicale*, November 1896; *Comptes rend.* January 1901.
[3] *Arch. de Physiol.* p. 333, 1897.
[4] *Hofmeister's Beiträge*, ii. p. 514, 1903.
[5] *Arch. ital. de Biologie*, xxxii. p. 210, 1900.

may conceivably be of direct value in bringing about the disappearance of intra-vascular blood-clots, such as are common in disease. The observations of Dastre,[1] who attributes fibrinolysis to the action of a fibrinolytic enzyme, accord best with the ascertained facts. Arthus and Huber[2] also confirm his statement that bacteria are not essentially concerned in the process. In experimental phosphorus poisoning not only may a firm clot rapidly pass into solution in the serum, but, as Jakoby[3] has shown, the serum in these cases possesses an intense fibrinolytic action on normal blood-clot.

Among the cardinal facts concerning blood when this liquid is considered as a whole, the knowledge that the blood on escaping from the body coagulates must be far more widely spread than are some other points of equal interest, such as the possession by the organism of a large excess of blood beyond the amount which is actually required for the performance of its functions, and that when the body-cells die or are diseased, as well as in a variety of conditions such as deprivation of food or on the introduction of foreign material into the circulation, the individual strives by every means in its power to maintain the constituents of the blood at their normal, absolute, and relative values. That the phenomenon of coagulation is a protective one is sufficiently obvious. When blood coagulates on the surface of or outside the body it is a normal process, just as clotting within the vessels is pathological. Cells detached from an organism may show instantaneous chemical and physical changes which are variously interpreted as signs of commencing death or signs of continuing life. In connection with this, recent papers by Vernon[4] and Schryver[5] may be consulted; and the latter has shown that, at any rate in the case of intestinal epithelial cells, disintegrative processes commence immediately these are detached from the body. But from a morphological point of view no cell or part of a cell detached from an organism exhibits such a rapid change as that shown by dying blood, and the rapidity of this change or rate of coagulation varies in different animals, and in the same animal under different

[1] *Comptes rend.* xlv. p. 995; *Arch. de Phys.* xxv. p. 661, 1894.
[2] *Arch. de Physiol.* xxviii. p. 857, 1896.
[3] *Zeitschrift f. phys. Chem.* xxx. p. 174, 1900.
[4] *Journ. of Phys.* xxxv. p. 53, 1906; *Zeits. f. allg. Phys.* vi. 1906.
[5] *Biochem. Journal*, vol. i. No. 3, 1906.

physiological conditions. In man it has been found that blood when shed either does not necessarily coagulate, as in hæmophilia, or does so slowly or rapidly. We must admit that the appearance of fibrin in shed blood is definite evidence of its altered state. This may take place within 10 to 20 seconds from the time blood is shed from a wound, for when examined as a stretched film at the temperature of the body in a moist chamber, a thread of fibrin some millimetres in length can be lifted from the film. In this interval of time, therefore, a gross change occurs—the appearance of fibrin, which does not exist in blood; and it is permissible to suggest that possibly blood commences to undergo other changes less easy to recognise *immediately* it leaves the body.

2. The Coagulation Rate of Human Blood

A knowledge of the rate of coagulation of human blood is considered by many clinical observers to be of diagnostic importance. It is difficult, if not impossible, to accurately ascertain this rate, since the results of different observers are conflicting, and this is due partly to the diversity in the methods employed as well as to the inherent difficulties in carrying out a series of successive experiments under exactly similar conditions. In this country most of the data have been obtained by the methods suggested by Sir Almroth Wright. The figures of this observer for normal human blood at a temperature of 20° C. are about 4 min. 50 sec. A series of between 300 and 400 observations made by this method at a temperature of 19·4° showed that in healthy women the average time was 7·75 min.,[1] though it may be as short as 5 or as long as 10 minutes. The average value obtained for normal men by an entirely different method at 20° is given at 7·8 min.[2] My own figures, given by another method but taken at the same temperature, average 8 min. 45 sec. Bürker[3] has devised yet a fourth method, and his recent figures show that the rate varies between 6 and 7·5 min. at 20°, although in the same individual, according to the time of day, amount of exercise, or kind of food, the rate of

[1] Douglas, *Brit. Med. Journ.* March 26, 1904.
[2] Brodie and Russell, *Journ. of Phys.* p. 403, 1897.
[3] *Pflüger's Arch.* cii. 1904.

coagulation may be as low as 5 minutes or as high as 14·5, a statement which I can confirm, though the method which was employed differed from that which he has described.[1]

3. State of the Subject at the Close of the Year 1880

The examination of the blood was essentially a study of those few early observers whose work at that time comprised not only the practice of medicine, but also all those subsidiary sciences bearing upon this subject which are to-day in some cases unfortunately so widely dissociated from medicine. During this time, which was succeeded by the period of humoral pathology, the contributions of Hewson and John Hunter form a definite starting-point in our knowledge of the coagulation process. The early discoveries of Hewson, those of Buchanan of Glasgow in 1845, those of Denis, who isolated a sero-fibrin, which he subsequently termed plasmine, from salted plasma—a substance which he proved, when mixed with water, gave a clot—down to the earlier work of Alexander Schmidt and Olaf Hammarsten on the conditions and factors influencing coagulation, have been already admirably described[2]; and our definite knowledge concerning the process of coagulation of the blood outside the body at the close of the year 1880 may, in the light of subsequent work, be briefly stated. Blood retained within the living vessels does not clot readily. Further, the plasma of blood clots in the same way and as readily as the whole mass of the blood. Contact with foreign material accelerates the clotting process or conceivably induces this, since blood remains unclotted for days when removed from the body with its containing vessel, or even if the wall of the vessel be replaced by a perfectly clean glass tube (Lister[3]). A definite protein substance—fibrinogen—can be precipitated from plasma, and solutions of this can yield fibrin. Moreover, fibrinogen exists as such in the plasma of normal living blood, since it can be thrown down as an insoluble body from living blood. The important proof of this fundamental statement is due to Fredrique, who, in 1877, repeated an old experiment of Hewson. He removed the jugular vein of a

[1] Buckmaster, *Morphology of Normal and Pathological Blood*, p. 212, 1906.
[2] Gamge, *Text-book of Physiological Chemistry*, vol. i. 1880.
[3] *Ibid* vol. i. p. 79.

horse, together with the contained blood, by ligaturing the vessel in two places. The operation was carried out aseptically, with a minimum amount of damage. The corpuscles settled down, and from the uncoagulated plasma an insoluble fibrinogen separated out at 56° C. A substance varying in activity, which is destroyed by heat, can be obtained from coagulating or coagulated blood. This is thrombin, or fibrin-ferment (Alexander Schmidt, 1872), which does not exist in circulating blood, and therefore coagulation does not occur during life; but thrombin appears when blood dies, and the surplus which is not used is found subsequently in the serum. The ferment is destroyed by boiling. It cannot be obtained as a pure product. We recognise the substance, as is the case with all ferments, by its specific action, that of converting a soluble fibrinogen into an insoluble fibrin. Under the microscope certain changes can be witnessed in a film of shed blood. From isolated highly refracting bodies, the blood-platelets, or from groups of these, strands of less refractive material can be seen to extend, form a network, entangle both the red and white corpuscles, and form a clot (Mrs. E. Hart, 1879[1]).

These statements embody the main facts known in 1880, and, though they may be found in all text-books of physiology, it is convenient to mention them here, since the workers of the past twenty-five years have been compelled to take these certain facts into account, and, indeed, no theory of coagulation can ignore them. Theories are not necessarily explanations, and the successive output of work in connection with coagulation has tended to the creation of a somewhat mystifying terminology and to the construction of innumerable theories. Apart from original papers, special contributions in English,[2] French,[3] and German volumes,[4] together with the admirable article by Morawitz,[5] have rendered it possible to sift out the less definite from those more definite facts which have thrown light on the nature of the coagulation process both within and outside the body.

The work of Alexander Schmidt himself, apart from that

[1] *Quart. Journ. of Microscopic Science*, xxii. 1882.
[2] E. A. Schäfer, *Text-book of Physiology*, vol. i. p. 168, 1898.
[3] Arthus, *La Coag. du Sang:* Paris, 1899.
[4] E. Schwalbe, *Untersuch. über Blutgerinnung*, 1890.
[5] *Ergebnisse der Physiol.* p. 307, 1905.

of his pupils of the Dorpat school, on the question of coagulation extended from 1860 to 1895. His conceptions as to the nature of the process underwent modification rather than destruction by other observers. Hammarsten in particular showed:

1. That all natural and artificial fluids containing fibrinogen can be caused to coagulate with fibrin-ferment.

2. That no other protein constituent of blood-plasma can be considered to exert any specific action in the process of coagulation, and that where such an action is apparent it is due to contamination of the substance with thrombin or fibrin-ferment, since the repeated purification of any protein which apparently induces a formation of fibrin abolishes this action, just as a temperature of 56° to 69° destroys any ferment adhering to the substance, though the substance itself is not apparently physically altered.

3. Fibrin-ferment converts fibrinogen into fibrin, a change which does not occur in living blood, since the ferment appears only in shed blood. In this phase of the coagulation process fibrinogen passes through a stage of soluble into insoluble fibrin, exactly as caseinogen passes into a soluble and then into an insoluble casein—though, as will be seen subsequently, the coagulation of fibrinogen-containing fluids by thrombin is in no sense paralleled by the action of rennin on caseinogen, as Arthus and Pagès originally believed.

4. Phases of the Coagulation Process

Since coagulation depends on the conversion of fibrinogen into fibrin in a series of steps by a ferment body which is non-existent during life, the phases of coagulation must be:

1. Formation of thrombin.

2. Conversion of fibrinogen into fibrin where a stage of soluble fibrin precedes the insoluble stage.

The grounds for this conception of the second phase—a belief that before any separation of fibrin occurs the fibrinogen suffers change—depend on some remarkable observations made by Hammarsten.[1] Although fibrinogen in hydrocele fluid[2] co-

[1] Quoted from *Jahresb. f. Tierchemie*, 1876.

[2] The spontaneously non-coagulable exudation found in a pouch derived from the peritoneum, which envelops the testis.

agulates at 60°, and an apparently identical fibrinogen in blood-plasma at 56°, the addition of fibrin-ferment to hydrocele fluid at once reduces the coagulation-temperature of this globulin to 56°. Ferment-free hydrocele fluid remains clear when thawed after freezing, whereas it appears turbid if treated in the same way after the addition of ferment. Since the same phenomena are seen when solutions of fibrinogen are employed instead of blood-plasma, it would appear that in the process of coagulation all the fibrinogen does not become fibrin, but a part of this, fibrin-globulin, which is a soluble protein coagulating at 64°, separates out. It is conceivable that, under the influence of thrombin, fibrinogen suffers a hydrolytic change into two bodies. Hammarsten believed that the fibrin-globulin was a remnant of the soluble fibrin, since the relative amounts of fibrin and fibrin-globulin produced by ferment action vary so enormously that a simple hydrolytic process is impossible. Recently Heubner[1] has stated that never more than half the fibrinogen in a liquid becomes fibrin, and inclines to the view that a hydrolytic action is exerted by the ferment. Huiskamp[2] has prepared solutions of fibrinogen by precipitation with sodium fluoride, and found that these, neither by heat nor by coagulation with ferment, yield any fibrin-globulin. This product, therefore, is not produced when blood coagulates, but is either pre-existent as such in plasma, or, as a separate body, may be loosely attached to fibrinogen.

The greater part of the work on the coagulation question since 1880 centres around the first phase of the process—the formation of fibrin-ferment, but it would appear to be of little use in this place to discuss the theories of those whose real work will be remembered for the establishment of incontrovertible facts.

5. The Rôle of Calcium Salts in the Process of Coagulation

Hammarsten, in 1875, had already recognised that the addition of calcium chloride to solutions of fibrinogen increased the yield of fibrin which could be obtained on coagulation; but it was essentially a discovery of Reynolds Green,[3] fifteen years later, that calcium salts are absolutely necessary in order

[1] *Archiv f. exp. Path. u. Pharmak.* xlix. p. 229, 1902.
[2] *Zeits. f. physiol. Chemie*, xliv. p. 182, 1904.
[3] *Journal of Physiol.* 1887.

that coagulation shall occur in salted plasma. When these are removed by dialysis, coagulation never occurs. He further proved that calcium does not act like a ferment, since it is incapable of coagulating either the fibrinogen in a hydrocele fluid or solutions of this globulin. Salts of strontium in a less degree act like salts of calcium.

Arthus and Pagès[1] decalcified blood by the addition of 1 gramme of potassium oxalate to a litre of dog's blood or by 1·5 to 3 grammes of sodium fluoride (Arthus[2]), or by a strong solution of alkaline soaps. The oxalated plasma clotted simply on the readdition of calcium chloride. Arthus and Pagès believed that oxalated plasma contained thrombin, since it could coagulate fibrinogen solutions; and since, together with other experiments, it was shown that the yield of fibrin was characteristically raised or lowered by the calcium content of the plasma, they concluded that calcium played a definite part in the second phase of coagulation, and that fibrin was really a calcium compound of fibrinogen. Pekelharing[3] repeated and extended this work, and showed that although oxalate-plasma does not contain thrombin, it does undoubtedly contain a zymogen or prothrombin, which on being activated by calcium becomes an enzyme—in fact, is thrombin. The zymogen of a salt-plasma can be thrown down together with the globulins, and such a precipitate contains thrombin, while a similar precipitate thrown down from oxalate plasma has no ferment power, but acquires this on the addition of calcium chloride. Therefore the zymogen termed prothrombin becomes thrombin on the addition of calcium, and when once at this stage its activity can be shown to be no longer destroyed by the subsequent addition of an oxalate. Arthus and Pagès considered that calcium essentially played its part in the second phase of coagulation; while Pekelharing demonstrated that during the first phase, when a formation of thrombin takes place, this was effected by salts of lime. In this way the ferment was fully formed from its zymogen stage. In the second phase of coagulation Pekelharing considered that the ferment handed on the lime, so that it was associated with fibrinogen, and thus yielded a calcium compound

[1] *Arch. de Physiologie*, xxii. p. 739, 1890.

[2] *Journ. de Phys.* 1901.

[3] *Intern. Beiträge f. Virchow's Festschrift*, 1891; *Deutsche med. Wochenschrift*, p. 1133, 1892.

of this globulin which was known as fibrin. In the theory of Lilienfeld,[1] though the existence of thrombin is accepted, the idea that it plays any important part in the process of coagulation is denied, and the behaviour of calcium in the process is regarded as identical with the views suggested by Arthus, for whom fibrin was a calcium compound of fibrinogen or a thrombosin curd. Alexander Schmidt, however, denied the specific action of calcium for either phase of the coagulation process; its presence in a coagulable fluid only favours the conversion of a zymogen into an enzyme and the formation of fibrin from fibrinogen. He therefore included calcium salts with these substances, which, like strontium, and possibly barium, exert a directly favouring influence on the rate and extent of the coagulation process.

These conflicting views have been completely explained by Hammarsten,[2] whose researches were directed towards affording a sufficient answer to two questions:

1. Are salts of lime absolutely necessary in order that fibrin-ferment shall act upon fibrinogen?

2. Can the whole coagulation process in its two phases occur in the absence of calcium salts?

Hammarsten showed that the total calcium content of blood is in two states. That portion which is in solution can alone be precipitated by oxalate, while another portion, which is probably united in organic combination with the proteins of the plasma, cannot be detached. The non-coagulability of oxalated plasma is therefore a matter related to that fraction of the calcium which can be precipitated. When oxalate-plasma is cooled to 0°, Pekelharing had already shown that a granular precipitate gradually separates out; and in proportion as this occurs, so does oxalate-plasma lose its peculiar property of coagulating on the addition of calcium chloride. It was a logical deduction that this separated material was essential for coagulation, and from the supernatant oxalated plasma Hammarsten precipitated a calcium-free fibrinogen by lime-free sodium chloride, and found that a calcium-free fibrinogen could be caused to clot on the addition of a calcium-free solution of thrombin. By this experiment it was conclusively proved that the presence of calcium is quite unnecessary for the conversion of fibrinogen

[1] See *Text-book of Physiology*, ed. by Schäfer, vol. i. p. 172.

[2] *Zeitschrift f. physiol. Chemie*, xxii. p. 333, 1896, and *ibid.* xxviii. 1899

into fibrin, and the latter body is certainly not a calcium compound of the former. A series of analyses confirmed this, since it was shown that the calcium content of fibrin is not higher than the fibrinogen which yielded it, and fibrin can be produced which contains only a trace of lime—0·007 to 0·0095 per cent. However, the absolute proof that calcium does take a specific part in the formation of fibrin-ferment, as Pekelharing believed, was confirmed by Hammarsten, since the material separated from cooled oxalated plasma contained a zymogen which could be directly and specifically activated by calcium salts, and induce coagulation of fibrinogen solutions, a property it did not possess unless these salts were added. Calcium salts or calcium ions, therefore, play an essential rôle in the first phase of the coagulation process by activating a zymogen. In this way thrombin, non-existent in living blood, originates in blood after it is shed. The absolute amount of calcium present is stated by Sabbatani[1] to be a negligible factor for the formation of thrombin. Only that portion which is in the ionic state is concerned in the process. The arguments for this view are that all physical methods which, like heat and cold, exalt or depress the amount of ionisation or affect the velocity of the ions, equally affect, accelerate, or retard the onset of coagulation. In the case of fluoride-plasma, which clots simply on the addition of water, the dilution may promote a sufficient degree of ionisation of the suspended lime salts.

6. Thrombin or Fibrin-Ferment

The interest attaching to this part of the question may be grouped around the three following facts:

1. The production of intra-vascular clotting by the injection of various tissue-extracts, and the development of a clot *in vitro* from non-coagulable or feebly coagulable liquids by the addition of tissue-extracts.

2. The discovery of Delezenne that the blood of those vertebrates which possess nucleated red corpuscles often does not spontaneously clot. Blood removed from birds such as the goose or pigeon with every aseptic precaution will yield a clear plasma which may actually commence to putrefy before it coagulates.

[1] *Arch. ital. de Biologie*, xxxix. 1, p. 333.

3. The recognition by Fuld and Spiro and by Morawitz that the term "thrombin" is applied to bodies which possess quite different properties.

Contrary to the results of previous observers in 1880, Edelberg,[1] a pupil of Schmidt, obtained intra-vascular clotting by the injection of solution of fibrin-ferment. In confirmation of the views of Schmidt that the fibrin-ferment was directly or indirectly a product of the leucocytes, Rauschenbach[2] in particular showed that not only leucocytes of the blood, but the cell-protoplasm of almost every tissue in the body, also yeast-cells (Wright), could markedly induce or accelerate coagulation in salted plasma, cooled plasma, and some, but not all, specimens of hydrocele and ascitic fluid. Tissues rich in nuclei possess this property not only *in vitro*, but when injected into the circulation—a fact which was definitely established by Foà and Pellacani,[3] who observed, moreover, that the tissue-extracts from the most varied sources, with the exception of the spleen, could induce intra-vascular clotting. Wooldridge[4] in particular confirmed these results, and in a remarkable paper showed that solutions of tissue-extract can not only cause a sudden death and produce extensive intra-vascular clotting, especially in the portal system, but that in dogs a definite dose per kilo body-weight can also produce conditions where the shed blood is found to be incoagulable. Further, a "Schutzimpfung auf chemischem Wege" could be demonstrated. This, the earliest example of an acquired immunity conferred by injection, was proved by showing that a small dose of injected tissue-extract, which Wooldridge subsequently termed B-fibrinogen, rendered a dog immune to a subsequent lethal dose of the same substance. These suggestive experiments, together with others on recognition of phosphorus in the extracts and his work on peptone-plasma led Wooldridge, like Rauschenbach, to speak of blood-plasma as protoplasma, and to hold that circulating blood contained within itself all that was essential for the coagulation process. The existence of fibrin-ferment played no part in his theory; both the coagulation and non-coagulation of blood was dependent upon the relations

[1] *Arch. f. exp. Path. u. Pharmak.* ii. p. 283, 1880.
[2] *Inaugural Dissert. Dorpat*, 1883.
[3] *Bir. clin. di Bologna*, p. 241, 1880.
[4] *Du Bois' Archiv*, 1881.

existing between the fibrinogen of blood and that other fibrinogen which originated from the tissues. He regarded the protoplasma as a living substance, capable, as he said, of being stimulated by such agents as carbon dioxide or weak acetic acid, so that a response or coagulation occurred.[1]

Tissue-extract is allowed by all observers to contain much nucleo-protein. The differences between this substance obtained from blood-plasma, leucocytes, or tissues such as the thymus or testis, and Schmidt's fibrin-ferment have been pointed out by many workers. Halliburton and Brodie,[2] among others, have insisted upon this difference. Whether nucleo-protein possesses a ferment action or whether in the preparation of this substance it becomes contaminated with thrombin, the same questions may be asked with reference to tissue-extracts, and investigations into the relations of these to the non-coagulating plasmata of birds have permitted a definite answer to be obtained, and at the same time enabled a large number of conflicting facts to be explained.

Although early observations on the coagulation of birds' blood showed that this quickly coagulated, Schmidt and Tiegel could not confirm this. The discoveries of Delezenne in 1897 that birds' plasma remained uncoagulated, that when the sedimented blood did coagulate the phenomenon started at the zone of leucocytes just above the red corpuscles and slowly extended upwards and downwards through the blood, and that both the blood and completely cell-free plasma quickly coagulated on the addition of tissue-juice from any source, have been confirmed by all observers. Among other work bearing on this subject that of Phisalix,[3] Fuld,[4] Fuld and Spiro,[5] Wright,[6] and Morawitz[7] may be mentioned here.

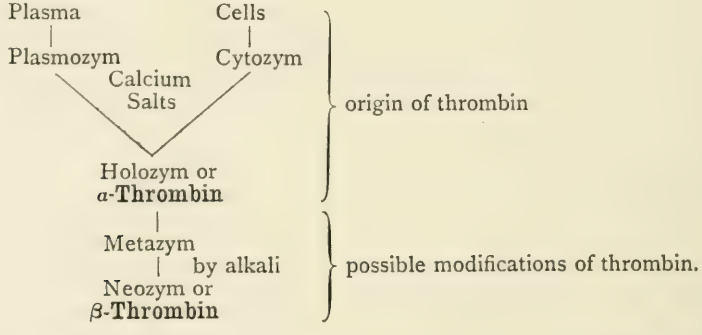
According to Fuld's view, whether thrombin action is similar or not to that of other enzymes, the formation of thrombin is due to the interaction of three factors which exist in the plasma of circulating blood or in plasma of unaltered blood outside the body. These are:

[1] Wooldridge, *Collected Papers.*
[2] *Journal of Physiology*, xvii. 1888.
[3] *Comptes rend.* li. pp. 881 and 885.
[4] *Centralbl. f. Physiologie*, p. 529, 1903.
[5] *Hofmeister's Beiträge*, v. p. 171, 1904.
[6] *Journ. of Phys.* xxviii.
[7] *Arch. f. Klin. Med.* lxxix.; *Hofmeister's Beiträge*, v. 1904.

1. Calcium salts.
2. A zymogen, or pro-ferment.
3. A substance which may originate from the corpuscles or from other tissues known as zymoplastic substance.

The pro-ferment which arises from the plasma is plasmozym; the substance originating from cells is termed cytozym. The interaction of these two and calcium ions yields a true thrombin or holozym. The subsequent destiny of this, when it is present in serum after the process of coagulation, is to pass into metazym, which is to be considered as a zymogen capable of passing into a ferment stage known as neozym, when it is activated by an alkali. The evidence for this conception, which may be expressed in the following scheme, can be better discussed with the help of later work, particularly that of Morawitz.

The formation and fate of thrombin would be indicated as follows (Fuld):



The cytozym of Fuld corresponds to the coagulating substance in tissue-extract; and since fowls succumbed in a few seconds to an intra-venous injection of cytozym, it was concluded that the formation of thrombin occurred in a circulating fluid which already contained calcium and plasmozym. The results of Arthus[1] obtained with fluoride-plasma are only in partial agreement with Fuld's observations. Non-coagulating fluoride-plasma is an excellent medium for ascertaining the existence of thrombin. Both blood-serum and Schmidt's thrombin produce coagulation in fluoride-plasma. This, however, remains non-coagulable when cytozym alone or calcium alone is added. It is a fair conclusion that tissue-extract therefore

[1] *Journal de Physiologie*, p. 887, 1901.

contains no thrombin, since it will not coagulate fluoride-plasma. However, a perfectly cell-free plasma from the bird, or peptone-plasma from the mammal, will clot perfectly with tissue-extract.

Blood allowed to flow about in the subcutaneous tissues coagulates much more readily than a control sample taken direct from the vessels,[1] and this, together with the observations already described, shows that, though tissue-extracts contain no thrombin, they do contain a substance termed by Morawitz thrombo-kinase, which is concerned in the production of thrombin.

Although Pekelharing and Delezenne believed that tissue-extract actually exerted its influence in virtue of its fibrin-ferment, Schmidt, Arthus, and Wooldridge denied this, and in a general way the action was attributed to the presence of nucleo-protein. In circulating blood this substance or zymogen is absent, but appears in shed blood. The prothrombin described by Schmidt he regarded as pre-existent and activated in the presence of lime salts by a zymoplastic substance, shed out of leucocytes. According to Schmidt, this is the substance found in tissue-extract.

In 1903 Morawitz proved that the zymogen or prothrombin of Schmidt and the zymogen described by Hammersten, Arthus, and Pekelharing are entirely different substances. They are both inactive stages of thrombin. One occurs in oxalate-plasma, and is activated by calcium salts and by nothing else. This is α-prothrombin, and is in part or entirely the material which separates out in cooled oxalate- or peptone-plasma. The other kind of zymogen, that of Schmidt, β-prothrombin, or Fuld's metazym, is peculiar to the serum separated from coagulated blood. It is non-existent in fluoride-plasma or in oxalate-plasma or in circulating blood. This form is not activated by calcium, but even in the absence of salts of this metal can be activated by weak acids or alkalies. The term metathrombin can be employed for this unactivated stage.

The development of thrombin from metathrombin is easily produced by weak alkali. When serum is exposed to the air for a few days, any thrombin present completely disappears, and the fluid possesses only metathrombin. In the following table these facts are made clearer:

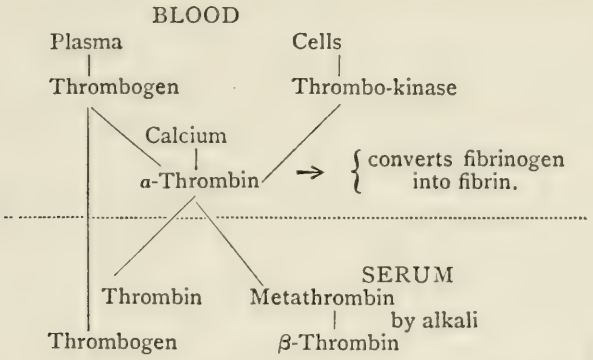
[1] Arthus, *Journ. de Physiologie*, iv. p. 281, and *Comptes rend.* liv. p. 136.

	Calcium.	α-Prothrombin, Zymogen	Thrombin	Metathrombin, Metazym, β-Prothrombin
Circulating blood . .	+	+	o	o
Oxalated plasma . .	o	+	o	o
Peptone-plasma . .	+	o	o	o
Fluoride-plasma . .	o	+	o	o
Serum from blood .	+	o	a little	+
Serum from any of the coagulated plasmata	+	o	a little	+

From the thymus, lymphatic glands, or the stromata of red corpuscles, a thermolabile substance can be extracted with a weak saline solution. This substance is, for the most part, thrombo-kinase, which soon becomes unstable in solution, though the extract, dried in a vacuum, yields a powder which only slowly changes. On fibrinogen solutions, even in the presence of lime, this extract produces no change; it is therefore not a thrombin such as occurs in serum. Exactly the same negative result follows when extracts from mammalian tissues are added either to solutions of fibrinogen or to oxalate- or fluoride-plasma, and it is immaterial whether the extracts are previously treated with calcium chloride or not.

On the other hand, the clotting of blood is accelerated, and the coagulation process in the plasma of birds, in peptone plasma, and sometimes in hirudin-plasma, forthwith commences on the addition of thrombo-kinase, provided calcium salts are present. The ferment-power of serum is also exalted by thrombo-kinase. From these facts Morawitz concludes that the development of thrombin results from the interaction of kinase with a body in coagulable liquids or in serum in the presence of calcium salts. This body is the substance recognised by Fuld as plasmozym, and may be termed thrombogen. This corresponds to *a*-prothrombin. Since *a*-prothrombin exists in fluoride-plasma as well as in all the non-coagulated but coagulable plasmata hitherto examined, the following conception of the coagulation process may be suggested. In the plasma of circulating blood a certain amount of fibrinogen, calcium, and thrombogen will be found. It does not coagulate, since thrombo-kinase is entirely absent, or if present in small amount is neutralised by anti-bodies in the blood. Solutions of thrombo-kinase or of substances capable of setting this free in the blood will forthwith give rise to thrombin, and be followed by intra-

vascular clotting. Outside the body the morphological constituents of the blood, and in particular the blood-platelets, yield kinase when these are brought in contact with non-living matter. The first phase of the coagulation process is thus initiated by the co-operation of thrombogen, calcium ions, and kinase.



The following table shows the accepted constituents of various plasmata on the suggested hypotheses of Morawitz, Fuld, and Spiro:

	Blood.	Serum.	Bird-plasma.	Oxalate-plasma.	Fluoride-plasma.	Peptone-plasma.
Thrombogen . .	+ ?	+	+	+	+	+
Kinase . . .	o	+ ?	o	+	+ ?	+
Thrombin . .	o	+	o	o	o	o
Metathrombin .	o	+	o	o	o	o
Calcium . .	+	+	+	o	o	+

Of the substances concerned in the production of thrombin, as described in the account just given, it would appear that very definite knowledge as to the sources or nature of thrombokinase and thrombogen in coagulable liquid does not exist. It is possible that kinase corresponds entirely or in part to the nucleo-proteid which apparently enters into the composition of the blood-platelets. Fluids such as goose-plasma, fluoride-plasma, or hirudin-plasma are devoid of kinase, while in oxalate or peptone-plasma this is present. On cooling these plasmata a granular precipitate occurs only in those which possess kinase, and this deposit is recognised in part to be nucleo-protein. As to the source or sources of thrombogen in blood there is

even more doubt. This body is not precipitated by cooling a liquid which contains it to 0° C., nor is it of a nucleo-protein nature. Suspensions of blood-platelets, obtained by centrifugalisation, have been regarded as yielding a supply of thrombogen. The prothrombin claimed by Schmidt to be present in circulating blood is apparently identical with thrombogen, and though this body is absent from plasma in cases of experimental phosphorus poisoning, the blood-platelets are present in large, if not excessive amount, and appear quite normal under the microscope. The repetition of old observations of Freund and Haycraft shows, moreover, that blood received in parafined or oiled tubes can be centrifugalised without clotting, and a perfectly cell-free plasma obtained, which clots on transference to a glass vessel.[1] But such a plasma contains no thrombin while in contact with paraffin, since the plasma can be decalcified. The contact-action of a foreign body therefore appears to be a deciding factor for the actual co-operation of those bodies which the researches of Morawitz have indicated as the probable factors in the formation of thrombin.

A large number of substances which possess toxic and ferment properties have been described as constituents of various snake-venoms. It is known that exceedingly minute amounts of the venoms of certain Australian and Indian snakes[2]—·0001 gramme per kilogramme body-weight—will cause intravascular clotting in dogs, cats, rabbits, and other animals. Such venoms contain veritable thrombins or fibrin ferments, which can clot blood *in vitro* (Lamb). They are destroyed in fifteen minutes by a temperature of 75° C. and impaired in power by filtration through a gelatine filter. Further, the ferment does not appear to be used up in the process of coagulation (C. J. Martin). That the clotting properties of venoms is dependent upon a formed thrombin appears certain. Their clotting power "is not due to their action upon prothrombins or thrombogens contained in plasma, as the presence of calcium ions is unnecessary." The venoms clot 10 per cent. oxalate plasma *in vitro* as rapidly as ·2 per cent., and their action is the same upon the following: citrate, fluoride and magnesium sulphate plasma, hydrocele fluid

[1] Bordet and Gengou, *Annal. de l'Inst. Pasteur*, 1903 and 1904.

[2] *Pseudechis porphyriacus*, *Notechis scutatus* (Australia); *Echis carinatus*, *Vipera Russellii* (India).

and solutions of fibrinogen prepared from blood plasma. The thrombins contained in venoms probably act as catalysts, and induce a formation of fibrin from fibrinogen. In this respect the behaviour of thrombo-kinase to birds' plasma, when coagulation is induced by the kinase effecting such a change in thrombogen in the presence of calcium that fibrin-ferment is formed, is in no way comparable to the action of venom on blood. For the thrombin of snake venom the rate of clotting varies with the amount of venom employed, so that the time multiplied by quantity of venom is constant.[1]

7. The Intra-vascular Injection of Peptone

From the fate which has overtaken nearly every theory which has been advanced to explain the coagulation process, it is permissible to predict that the one advanced by Morawitz will eventually prove to be only a provisional working hypothesis. If thrombo-kinase can be derived from any one or all of the morphological bodies in blood, it is difficult to understand why, during life, small quantities of this do not exist in circulating plasma. That the leucocytes at any rate disintegrate while circulating is almost certain. Contrasted with the duration of the life of the erythrocytes, the polymorphonuclear leucocytes survive only a few days. Any liberated kinase will play a part in the production of thrombin, and this will be rendered inactive by some anti-body or, what is improbable, since it is somewhat stable, changed into another form. It is also conceivable that any liberated kinase may be also neutralised, so that no formation of thrombin is possible.

The existence of thrombin cannot be demonstrated in many extra-vascular plasmas, though both proteolytic enzymes and anti-enzymes have been described in blood-serum and considered to be existent in plasma. Bordet and Gengou have shown that neutral salts hinder or suppress the formation of thrombin from its precursors, and in stronger concentration entirely abolish the activity of any thrombin which may be present. Similarly, the cooling of plasma acts essentially in the first manner. Arthus arranges the bodies which restrain coagulation into two groups :

1. Those substances—of which peptone, or rather deutero-

[1] C. J. Martin, *Journ. of Phys.* xv. 1894 ; xx. 1896 ; xxxii. 1905.

albumose, is the type—which exert specific action only when they are introduced into the circulating blood. The reaction of the organism, consequent upon intravenous injection, is essential, in order that substances restraining coagulation shall arise.

2. Substances which act independently and directly upon blood both inside and outside the organism.

Various animals react differently to peptone injection, and individuals of the same species require various amounts of peptone per kilogramme body-weight. To obtain blood which, when shed, shall be completely incoagulable, the injection must be made into the circulation with rapidity, and the animals should be fasting. Contejean states that when peptone is introduced into the peritoneal cavity it has no effect on the coagulability of blood. Certain snake-poisons, thrombo-kinase, or nucleo-protein, and the artificially synthesised celloids of Grimaux, which give many of the colour reactions of protein bodies, will all in various doses induce intra-vascular clotting. Albino rabbits and the Norwegian hare in winter-time are generally regarded as immune to an injection of the last two bodies (Pickering), but, as E. H. Starling has pointed out, the statement is inaccurate for albino animals.

The injected peptone is a digestive product, obtained by the action of trypsin. It is a mixture of albumoses and probably other less-known substances. Pick and Spiro consider that a heat-resistant body, peptozym, which is without influence upon extra-vascular blood, is the essential agent which affects intravascular plasma. The behaviour of peptone-plasma is inconstant. It may clot after a time spontaneously, but may do so quickly or not at all. Witte's peptone, in doses below 2 centigrammes per kilo body-weight, hastens coagulation, but above this dose it exerts a retarding influence (W. M. Thompson[1]). Water, weak acetic acid, or a stream of carbon dioxide, induces coagulation in peptone-plasma, even when this is free from leucocytes (Wooldridge). From a mass of conflicting statements it would appear that peptone-plasma contains thrombogen, kinase, and calcium ions, but no thrombin. It is probable that, like normal plasma which contains some anti-thrombin, an excess of this substance is present, and its development is due to a reaction of the organism following the introduction of peptozym. That anti-kinase is also present is doubtful, since

[1] *Journal of Physiology*, vol. xx. 1896.

the plasma easily clots on the addition of tissue-extract. The development of anti-thrombin is apparently bound up with the function of the liver. If this is shunted out of the circulation, peptone, on injection, is without influence on the coagulability of the blood, though if only the lymphatics of the organ are ligatured, peptone, as Starling and Delezenne have shown, still produces its effect. The artificial perfusion of the liver, but of no other organ, with a fluid containing peptone, yields an anti-coagulant substance, not because this is formed directly from the peptone, but from the liver-cells as a specific product of reaction. A complete explanation of the effect of peptone is at present not possible. It resembles the plasma of birds, except that in these animals thrombo-kinase is absent. An injection of peptone into the goose yields a blood with a marked amount of anti-thrombin. Such blood clots easily with kinase, but with difficulty on the addition of thrombin or goose serum. In this respect the effects of peptone injection are less marked in birds than mammals (Fuld).

8. The Intravenous Injection of Thrombo-Kinase

An injection of kinase may, according to the animal and the dose, yield a blood from which a kinase-plasma may be obtained that clots slowly or not at all. The introduction of this substance, as already mentioned, may cause intra-vascular clotting, but the organism, as the observations of Wooldridge, Groth, and Wright have shown, undoubtedly can cope with and neutralise the effects that would occur if thrombin, as such, circulated in the blood. When clotting is induced, as Wright[1] first pointed out by experiments on animals rendered either apnœic or asphyxiated, the presence of carbon dioxide in the blood is one of the deciding factors. Essential differences have been pointed out by Boggs between peptone- and kinase-plasma. Dilution with water, addition of acid, or addition of calcium chloride does not induce the latter to coagulate. When the action of thrombin upon kinase-plasma is contrasted with its behaviour towards peptone-plasma, it can be seen that coagulation occurs readily in the former, but with difficulty or not at all in the latter, while other experiments have shown that

[1] *Journ. of Physiol.* xii. 1891.

kinase-plasma does not readily clot when treated with thrombokinase. Moreover, Wooldridge some years ago pointed out that kinase-plasma could be easily coagulated by the addition of peptone-plasma. The plasmata obtained by injection are therefore dissimilar in many respects; in peptone-plasma an anti-thrombin predominates, in kinase-plasma an anti-kinase.

9. Substances which directly Hinder Coagulation both inside and outside the Body

Substances such as hirudin, which is the active principle secreted by the salivary glands of the medicinal leech, a similar body obtained from the heads of other blood-sucking worms, such as ankylostoma caninum, as well as the virus of many thanatophidia, together with anti-bodies, such as those described by Pugliese[1] and extracted by him from the blood and almost every organ of the body except the brain, directly check the coagulation of blood both inside and outside the organism.

The anti-body or bodies are, according to Pugliese, chiefly obtained from the liver, kidneys, and muscles. They are not destroyed by heat, but are diffusible. His results may be easily seen in the following table:

						Clot.
Plasma	+	Anti-bodies	+	Ca.	=	o
„	+	„	+	H_2O	=	o
„	+	„	+	Serum	=	+
„	+	„	+	Kinase	=	+

It would seem therefore as if both anti-kinase and anti-thrombin were capable of being yielded by nearly all the tissues of the body. The inner coat of arteries, which might have been expected to form anti-bodies during life, have, however, been shown by Loeb[2] to yield no anti-bodies of any kind.

The term "hirudin" was introduced by Jakoby and Franz. This substance appears as yellowish scales soluble in water with a slight turbidity, and the activity of the solution is not destroyed by boiling. Its activity is such that a definite amount, 0·01 gr., will maintain 7·5 cc. of blood fluid outside the body. The researches of Fuld and Spiro show that hirudin can undoubtedly neutralise thrombin both within and outside the

[1] Quoted from *Centralbl. f. Physiologie*, 1906.

[2] *Virch. Archiv.* clxxvi. 1904, and *Hofmeister's Beiträge*, v. 1904.

body, but only in certain proportions. Pekelharing considers that its action depends upon hindering the formation of proferment, *i.e.* kinase, from the corpuscles of blood, but this is improbable, since in hirudin-blood all the corpuscles are particularly well preserved, and on cooling the plasma no separation of any granular precipitate can be noticed. The differences between hirudin-, peptone-, oxalate-, and fluoride-plasma can be seen in the following table, from which it may be concluded that in the case of hirudin this substance does not remove lime nor neutralise any kinase:

		Clot.
Peptone-plasma	+ Weak acetic	= +
„	+ Water	= +
Oxalate-plasma	+ Calcium	= +
Fluoride-plasma	+ Calcium	= o
„	+ H_2O	= +
Hirudin-plasma	+ H_2O	= o
„	+ Calcium	= o
„	+ Acetic acid	= o
„	+ Kinase[1]	= + or o

Hirudin, therefore, exerts its effects by neutralisation of any thrombin which may appear. Fuld and Spiro have also shown that no neutralisation-ratio exists between thrombo-kinase and hirudin. Schittenhelm and Bodong do not hold this view, since a solution of fibrinogen containing calcium salts is not prevented from clotting by hirudin, and in the case of hirudin-blood the action is set aside by a definite amount of thrombo-kinase. These authors also draw attention to the fact that a suspension of blood-platelets, which after a time spontaneously gelatinises, does not pass into this state when hirudin is present. Hirudin-blood has also been shown to be markedly resistant to putrefactive change, possibly owing to the liberation of alexines from the leucocytes.

Among snake-poisons, the anti-coagulant effect of cobra-virus is set aside by the anti-venom prepared by Calmette, and Morawitz has shown that the action of the venom is dependent on the existence of an anti-kinase.

[1] Depends on the amount of thrombin produced in relation to amount of hirudin present.

10. Morphology of Blood-coagulation

Recent papers of Loeb on the process of coagulation in vertebrate blood, those of Ducchesi, Bürker, and more recently Nolf, deal with this part of the subject. Loeb, whose view corresponds in some respects to that of Nolf, who worked with the blood of vertebrates, distinguishes two distinct phases in coagulation:

1. The stage of agglutination of leucocytes in arthropoda and crustacea, and of blood-platelets in mammals.

2. The formation of fibrin from this agglutinated stage.

Loeb finds that peptone does not hinder clotting in the lobster, neither does kinase from vertebrates induce coagulation in the blood of invertebrates. In the blood of *Limulus* he states that a fibrinogen coagulable at 56° does not exist, and that this temperature does not interfere in any way with the first stage of coagulation. On dilution with water the blood of this animal shows agglutination of leucocytes, and the remaining liquid clots easily on the addition of muscle-extract from the same animal.

The macroscopic appearances on glass in shed blood to which Ducchesi[1] has drawn attention as the first alteration which blood undergoes outside the body, is due to an accumulation of platelets, and is a phenomenon entirely absent in peptone or hirudin-blood. This is also a definite agglutination process. Every observer, however, has seen that the bodies termed "platelets" are preserved in both these fluids. But agglutination rarely occurs. Regarded purely from the morphological point of view, it is possible that both inside and outside the body any damage to the blood-plasma is followed firstly by production and secondly by agglutination of previously non-existent platelets,[2] and then this stage is succeeded by the subsequent appearance of fibrin. As far as the relation of these phases to the coagulation process is concerned, this view is similar to that recently proposed by Bürker.

[1] *Hofmeister's Beiträge*, iii. p. 378.

[2] I am able to confirm a recent observation of Marino (*Comptes rend.* No. 4, 1905), who observed that when rabbits' blood is received into so much absolute alcohol that this is only slightly diluted, no platelets can be demonstrated to exist. It is inconceivable that they are destroyed, since the same blood mixed with 95 per cent. alcohol yields an abundant supply. This experiment, therefore, lends no support to the view that platelets exist in undamaged circulating blood.

Nolf's[1] recent contributions can scarcely be said to simplify the coagulation question. Besides the salts of calcium, he recognises three protein bodies which are concerned in the formation of a clot:

1. A protein derived from the leucocytes, termed leucothrombin.

2. One found in the liver—hepatothrombin.

3. Fibrinogen, which is formed by the activity of the cells of the same organ.

The interaction of the first two with calcium yields fibrinferment. Thrombin arises by the formation of an envelope of hepatothrombin around fine particles of leucothrombin, which appear early in a clear, spontaneously coagulable liquid. This process is followed by gelatinisation of the plasma, owing to the further aggregation of another layer of fibrinogen upon the thrombin. Fibrin, therefore, originates from an agglutination process which becomes established in a liquid plasma which at first shows no morphological constituents, and the whole phenomenon of coagulation is to be regarded as one of agglutination which takes place in a perfectly clear plasma which is originally devoid of any morphological constituents.

[1] *Archiv Internat. de Phys.* iv. 1906.

PROTEIN METABOLISM

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In an earlier number of this journal Dr. F. Gowland Hopkins, of Cambridge, in an interesting paper, drew attention to the important and interesting subject of the utilisation of the protein moiety of the food. As he fully discussed the subject in its general bearings, I do not wish to do more than draw attention to the advances which the more recent work has made in our knowledge of the various changes which proteid undergoes before absorption, and, in a brief fashion, to refer to the probable fate of the various fractions after such absorption has taken place.

Before, however, we can gain any insight into this profound problem, the question of how, and in what form, the protein is absorbed must be definitely determined.

Formerly it was universally held that protein was absorbed after digestion in the form of albumoses and peptones, which were immediately converted into albumen, and the whole subject was then dismissed. The work of the past few years has shown that this cut-and-dry statement is by no means justified, and that the problem is of a much more complicated nature.

Before dealing with the absorption of the hydrolysed, *i.e.* the digesten proteid, it would be well to consider the absorption of unaltered, undigested, or native proteins, as they are called.

The work of many investigators, both in Germany and elsewhere, has demonstrated beyond cavil that native protein injected into the blood stream can be utilised to a certain extent—indeed, the recent work of Sollmann and Brown[1] has shown that under favourable conditions egg albumen injected intravenously can be well utilised; in many instances only a

[1] Sollmann and Brown, *Journ. of Exper. Med.* 6, 407.

mere trace is excreted through the kidney. How and where this utilisation takes place is as yet unknown.

The classical work of Miescher on the Rhine salmon demonstrated the fact that the body can change one protein substance into another without the intervention of the intestinal wall. This observer stated that after the fish entered the fresh water they ceased to take food, and yet in spite of this, great anabolic changes took place in the salmon body. These changes consisted in the marked growth of the generative organs, which was shown to take place at the expense of the muscular system.

As regards the absorption of native proteins from the intestine, the old work of Voit and Bauer,[1] which showed that absorption of undigested proteins such as serum and egg albumen could take place, has been extended and confirmed by Heidenhain,[2] Friedländer,[3] Waymouth Reid,[4] and others. Ascoli,[5] with the newly introduced biological methods of testing for the presence of proteins, has been able to prove that the protein taken up is unchanged, and can give the precipitin reactions significant of the protein introduced. The absorption of unchanged protein can hardly, however, be said to occur under normal conditions. In the experiments referred to the protein was introduced in excess into the intestine. This absorption would seem also to be dependent to some extent on an increased permeability of the intestinal wall, such as is found in the very young. Another factor which plays an important part in this form of absorption is the presence of water, or salt solution. Friedländer (*l.c.*) has shown that after all the water or salt solution has been taken up, absorption of the protein, to all intents and purposes, comes to a standstill.

The objection which has naturally been raised to these various experiments is that, although the observers say that the intestine used in the experiment was thoroughly washed out previous to the introduction of the protein, yet there may have been sufficient trypsin left to act on the protein, and

[1] Voit and Bauer, *Zeit. f. Biol.* v. 536, 1869.
[2] Heidenhain, *Pflüger's Archiv*, 56, 1894, 579.
[3] Friedländer, *Zeit. f. Biol.* 33, 1896, 264.
[4] Waymouth Reid, *Phil. Trans.* Ser. B. 192, 1900, 21.
[5] Ascoli and Vigan, *Zeit. f. physiol. Chem.* 39, 1903, 283.

thus bring about its absorption in what might might be termed a natural fashion. This objection can, however, be dismissed (1) owing to the rapidity of the absorption which has been observed, and (2) because the amount of enzyme left after the cleansing of the intestine must be very small indeed. The figures obtained by Heidenhain (*l.c.*) and Waymouth Reid (*l.c.*) showed that the intestine could deal with quite large amounts of serum in a comparatively short time. It is impossible to understand how the trace of trypsin, supposed to be present, could bring this about, more especially as trypsin does not act by any means rapidly on this form of protein in its native state.

How then is the protein normally absorbed? Protein foodstuffs after ingestion are first of all acted on in the stomach by the pepsin present; here, however, under normal conditions decomposition is not fully carried out. This gastric digestion seems to aid the final action of the trypsin in some way other than the mere mechanical aid given by rendering the more or less solid masses of foodstuff suitable for the rapid attack of the trypsin and other intestinal enzymes. Abderhalden and Fischer[1] have shown that protein stuffs which have been digested first by pepsin and then by trypsin, yield, *in vitro* at least, a much smaller amount of polypeptide substance than do the same proteins when they have been subjected to the action of trypsin alone. Further, Abderhalden and Rona[2] have demonstrated, by feeding experiments on mice, that the digest which arises from the combined action of pepsin and trypsin has not the same power of preventing loss of nitrogen—in other words, has not the same food value as the products yielded by trypsin alone. On the other hand, from the experiments of Czerny and others one might conclude that the stomach is a quite unnecessary organ. They have shown that animals from whom the stomach has been practically completely removed can not only exist, but after they have accustomed themselves to the new conditions can actually thrive. The food from the stomach is passed on into the small intestine, where it is more or less completely broken down by the action of the trypsin into simpler bodies—into albumoses, peptones, and bodies which no longer give the biuret reaction. The

[1] Abderhalden and Fischer, *Zeit. f. physiol. Chem.* 40, 1904, 215.
[2] Abderhalden and Rona, *Zeit. f. physiol. Chem.* 42, 1904, 528.

albumoses and peptones, as Cohnheim[1] has shown, can be resolved into simpler bodies—the amino acids, by the action of a ferment (erepsin) discovered by him in the intestinal mucous membrane. This ferment, it may be stated, does not attack native proteins with the exception of casein.

The important question which is now awaiting solution is whether it is necessary that the protein be so far decomposed before it can be utilised by the body tissues for transformation or resynthesis into the complex body proteins. Many observations on this point have been carried out by investigating the contents of the intestinal canal of animals killed a few hours after a full meat meal. This method, however, has the serious drawback that both the decomposition of the protein and the absorption of the products go constantly on together, thus no time is allowed for any accumulation of the products in the gut. Further, it is quite possible that some of the products formed may be more readily absorbed than others, and in this way the material which is eventually obtained from the intestine may not give a true indication of the real nature of the decomposition. Kutcher and Seemann[2] have carried out such experiments, and have demonstrated in the intestinal contents the presence of quite a number of the well-known decomposition products. They were able to isolate leucin, tyrosin, lysin, and arginin, thus both mono- and di-amino acids. Abderhalden[3] has also investigated this question along the same lines. He was able to isolate from the intestinal contents by Fischer's ester method alanin, leucin, glutamic and aspartic acids, and perhaps traces of *a*-proline and phenyl-alanin. Tyrosin was also found. Some polypeptide was also present, and yielded on hydrolysis with acid glycocoll, *a*-proline, and phenylalanin.

A very important point was made a few years ago when O. Loewi[4] proved that animals which were fed on the decomposition products of proteins, obtained by prolonged digestion with trypsin until complete disappearance of the biuret reaction, could be kept alive. Not only did he keep the animals alive with these products as the sole source of their supply of

[1] Cohnheim, *Zeit. f. physiol. Chem.* 33, 1901, 451.
[2] Kutscher and Seemann, *Zeit. f. physiol. Chem.* 34, 1902, 528.
[3] Abderhalden, *Zeit. f. physiol. Chem.* 44, 1905, 617.
[4] Loewi, *Schmiedeberg's Archiv*, 48, 1902, 303.

nitrogen, but they actually retained a percentage of the nitrogen given. An interesting fact was brought out in the course of these observations as regards the calorific value of the decomposition products. A calorimetric estimation of one gramme of products was carried out by Rubner, with the result that this amount was found to equal 4·599 cal., thus very similar to the figure for albumen. There must be given, along with these decomposition products, an abundant supply of carbohydrate food in the form of sugar and starch. Recently Lüthje[1] has proved that it is absolutely essential that the animal get carbohydrates, and further, that these carbohydrates cannot be replaced by fat in the diet. The fact that Lesser,[2] who denied the correctness of Loewi's work, only used fat and no carbohydrates, probably fully explains his negative results. Loewi's results have been fully confirmed by other workers, such as Henriques and Hansen, Abderhalden, and others. Henriques and Hansen[3] not only were able to confirm Loewi's results, but showed further that only a fraction of the products produced by pancreatic digestion could keep the animal alive. They found that those products which were soluble in alcohol sufficed, whereas the insoluble fraction was quite inert. Again, they showed that the part of the digest not precipitated by phosphotungstic acid, that is the so-called monamino acid fraction, had the nutritive value; here, again, the part precipitated was of no use as a food. This statement, that a part merely of the products suffices as a food, has been fully confirmed by Lüthje (*l.c.*), always supposing, however, that there be a plentiful supply of carbohydrate food in addition. In this respect the animal body closely approximates the vegetable kingdom, where it has been shown that carbohydrates play a very important part in the protein synthesis. Abderhalden[4] and his co-workers have also confirmed and extended Loewi's results. As I have already mentioned, differences were found between the nutritive values of protein digested by trypsin alone and by pepsin followed by trypsin. They, like Henriques and Hansen, found that although the products arising from enzyme action were effective, those which were

[1] Lüthje, *Pflüger's Archiv*, 113, 1906, 547.
[2] Lesser, *Zeit. f. Biol.* 45, 1904, 497.
[3] Henriques and Hansen, *Zeit. f. physiol. Chem.* 43, 1904, 417.
[4] Abderhalden and Rona, *Zeit. f. physiol. Chem.* 44, 1905, 198.

obtained by hydrolysis with acid were of no use. In a recent paper, it is true, Henriques and Hansen[1] have shown that the acid products can act to some extent as sparers of protein. How, then, do the acid hydrolysis products differ so much in this vital action from the products obtained by digestion with ferments? Is it that by the more active boiling acid products which are of absolute necessity for the maintenance of life are too far broken down, or is it perhaps due, in part at least, to the racemisation which takes place? One series of bodies which are known to be formed in tryptic digestion, and not during acid hydrolysis, the polypeptides—bodies formed by the linking together of two or more amino acids—cannot play any part, as it has been shown (Henriques and Hansen) that when they were removed by precipitation the monamino acids which were left sufficed. Whatever the cause, the main fact still stands that animals fed with acid hydrolysis products perish, whereas animals fed with ferment digest products not only live, but may even thrive on them.

As regards the statement that the products of protein digestion were absorbed in the form of albumoses and peptones, it rested mainly on the work of Salvioli,[2] carried out many years ago. This worker stated that if he isolated a loop of intestine, and into it put the products in question (albumoses and peptones), on perfusion with blood he found that they disappeared. Further, Salvioli was unable to detect them in the blood used for perfusion, therefore he concluded that the absorbed products were regenerated to more complex bodies before they reached the blood. Recently the writer, working in conjunction with Dr. Leathes,[3] repeated the work of Salvioli, and found that albumoses and peptones introduced into the loop of intestine did indeed disappear, but the interpretation, supported by analytical data, was quite other. We found that the peptone, etc., introduced into the loop was merely split up into simpler bodies which do not give the biuret reaction, in all probability due to the action of the ferment erepsin. If it be due to the erepsin, then this ferment could not have acted intracellularly as Cohnheim holds, as so far as we could

[1] Henriques and Hansen, *Zeit. f. physiol. Chem.* 49, 1906, 113.
[2] Salvioli, *Du Bois Reymond's Archiv*, 1880, Suppl. p. 95.
[3] Cathcart and Leathes, *Journ. of Physiology*, 33, 1906, 462.

estimate no absorption took place. Of course, as Salaskin[1] and Cohnheim himself have shown, there is a small quantity always to be found free in the lumen of the gut.

Very contradictory statements exist as to whether albumoses and peptones are ever normally present in the blood. One had come to believe that there was no evidence of their presence in this fluid, but a year or two ago definite statements that albumoses had been detected were published by well-known workers. The results were not always positive, however, even with blood examined during the absorption of proteins. Embden and Knoop,[2] and later Langstein,[3] stated definitely that they had managed to prove that albumoses were among the normal constituents of normal blood. This statement has been denied by Abderhalden and Oppenheimer,[4] these workers maintaining that it is not a normal constituent even under the most favourable conditions. For instance, three dogs which had been starved for several days were given a full meat meal, and then, six to eight hours later, were killed. On examination no trace of a biuret-giving substance was found in the blood thoroughly freed from its protein. They suggest that the positive results have been probably due to traces of protein left owing to imperfect coagulation. Howell,[5] in a recent paper, came to the same conclusion after having carried out many experiments with a fairly large series of animals at different periods of digestion. The method he employed was one of dialysis through membranes permeable to albumoses and peptones, but not to the serum proteins.

If the absorption takes place in the form of the amino acids, which are soluble bodies, one would expect on examination of the blood to find, after a full protein meal, a very definite increase in the amount of non-precipitable or soluble nitrogen. This, however, does not turn out to be the case, as Dr. Leathes and the writer (*l.c.*) have shown that there is an increase, but it is not very great. Of course, when one considers the rapidity of the blood flow through the intestinal vessels, it is perhaps rather to be wondered at that any evidence

[1] Salaskin, *Zeit. f. physiol. Chem.* 35, 1902, 419.
[2] Embden and Knoop, *Hofmeister's Beiträge*, 3, 1902, 120.
[3] Langstein, *Hofmeister's Beiträge*, 3, 1902, 373.
[4] Abderhalden and Oppenheimer, *Zeit. f. physiol. Chem.* 42, 1904, 153.
[5] Howell, *Amer. Jour. of Physiol.* 17, 1906, 273.

of increase at all was obtained in the comparatively small amount of blood which could be examined. Abderhalden (*l.c.*) attempted to prove the presence of amino acids in the blood after a full proteid meal, but without success. The method which he employed was the very excellent one devised by Fischer, in which the amino acids, even when present in small amount, form crystalline compounds with β-naphthalinsulphochloride. Howell (*l.c.*), by using this same method in conjunction with a process of dialysis, has reported that he has been able to detect small amounts of amino acids in the blood taken from dogs at the height of digestion; more, further, in the portal blood than in blood taken from the general systemic circulation. Even in the blood of dogs which had fasted for a considerable number of hours, a positive reaction could still be obtained.

A point in favour of the full breakdown of the protein molecule was given in a piece of work by Abderhalden and Samuely,[1] in which an attempt was made to affect the constitution of serum protein. A horse was bled to an extreme degree, and then was fed with a protein obtained from wheat—gliadin. This protein differed very markedly from the constitution of the serum protein, especially in its content of glutaminic acid (gliadin has 36·5 per cent. glutaminic acid, whereas serum albumen has only 7·7, and serum globulin 8·5). When the horse on the gliadin diet had regenerated its blood to a large extent, it was again bled, and the constitution of the newly formed serum proteid investigated. Results, as regards the alteration of the internal constitution of the body protein by the nature of the food protein, were quite negative. The serum protein obtained resembled that formerly present in every respect. The fact, too, that Howell (*l.c.*) was able to detect amino acids, but no trace of albumoses or peptones in the blood, seems to lend support to the view that the hydrolytic changes in the intestine are pretty thorough.

In what form, then, does the absorbed protein reach the tissues proper? Is it absorbed as simple amino acids in solution absolutely unchanged? In all probability not to any great extent, as is shown by the slight increase of the nitrogen in the blood. Of course, it might be absorbed by means of the leucocytes, as suggested by Hofmeister, and that owing to

[1] Abderhalden and Samuely, *Zeit. f. physiol. Chem.* 46, 1905, 193.

the methods of analysis employed it was not detected. On the other hand, these amino acids can lose their nitrogen fairly readily through the action of the body tissues, as has been demonstrated by the work of Jacoby[1] and of Lang.[2] Lang showed that if one mix various amino acids with chopped-up animal tissue, such as liver, spleen, intestine, etc., the amino groups are split off. For instance, glycocoll was found to give up its nitrogen freely when mixed with liver, but even more freely when in contact with intestinal tissue. Tyrosin could be attacked by the liver, but only with difficulty. Leucin, on the other hand, was fairly readily decomposed by that organ, and so on with other amino acids. Thus it may be that the absorption of the decomposition products is in the form of bodies nitrogen free, as acids of the fatty acid series such as lactic acid. In favour of this view, perhaps, the work of Nencki and his co-workers may be cited. Nencki, Pawlow, and Zaleski[3] showed that the blood taken from the portal system contains three to four times more ammonia than the systemic arterial blood. This is extremely well marked where the animal has previously been fed on a meat diet. This ammonia might be regarded as that split off from the amino acids during or after their course through the intestinal wall, and which, for the most part, is being hurried to the liver, there to be converted into urea for excretion. It must be remembered, however, that according to Horodynski, Salaskin, and Zaleski[4] starvation has practically no effect on the amount of ammonia in the arterial blood, and that there is only a very slight reduction in the amount present in the portal blood.

Others, again, hold that there is no such simple absorption, but that as soon as they are absorbed the products of digestion undergo synthesis, and in this reperfected form reach the body circulation. As regards this question of regeneration of a coagulable protein, Dr. Leathes and the writer (*l.c.*) were quite unable to detect any such increase, although this question was carefully gone into. Neither by an estimation of the total nitrogen before and after absorption nor by controls carried out by observations on the amount of hæmoglobin, were we

[1] Jacoby, *Zeit. f. physiol. Chem.* 30, 1900, 149.
[2] Lang, *Hofmeister's Beiträge*, 5, 1904, 321.
[3] Nencki, Pawlow, and Zaleski, *Schmiedeberg's Archiv*, 37, 1896, 26.
[4] Horodynski, Salaskin, and Zaleski, *Zeit. f. physiol. Chem.* 35, 1902, 246.

able to prove an increase. Kutscher and Seemann (*l.c.*) were unable, in their experiments, to find the crystalline products which they isolated from the contents of the intestine during digestion, either in the intestinal wall or in the blood. They discovered, however, in the wall of the intestine biuret-free extractive bodies which, on treatment with boiling acids, yielded leucin. They drew the conclusion from this observation that the leucin (and probably other crystalline products) absorbed from the canal is linked up with some other substance or substances into bodies of a more complex nature in the intestinal wall. Embden and Knoop (*l.c.*) were quite unable to prove that any synthetic action ever took place in the walls of the small intestine, when working, at any rate, with surviving gut outside the body. This is quite the reverse of what Glaessner[1] claimed to have found in the case of the gastric mucous membrane. This worker stated that, as Hofmeister[2] had previously found, if the albumoses present at the time of digestion be allowed to remain in contact with the isolated mucous membrane of the stomach, they for the most part disappear as such, and that in their place appears a coagulable protein body. Concerning the nature of this protein Glaessner says nothing, but holds its formation to prove that by the alteration of the albumose a synthesis has taken place. Objections have been raised to this work, and Cohnheim has held that the results noted by Glaessner may be due to differences in the case of coagulability of absolutely fresh protein of the mucous membrane, and of that which has been out of the body for some little time. Salaskin[3] offered as an explanation that the increase of protein may simply be due to a normal restitution of the cell protoplasm after its activity in ferment production.

Reference may be here made to a change which has been declared by some to be a true resynthesis—namely, to the formation of anhydride albumen, or plasteïn as it has been called. The curious property possessed by rennin of producing a precipitate—the plasteïn—in solutions of albumoses or peptones was first brought to notice by Okunew[4] in a Russian

[1] Glaessner, *Hofmeister's Beiträge*, 1, 1901, 320.
[2] Hofmeister, *Schmiedeberg's Archiv*, 19, 1885, 1.
[3] Salaskin, *Zeit. f. physiol. Chem.* 35, 1902, 419.
[4] Okunew (quoted by Sawjalow, *Pflüger's Archiv*, 85, 1901, 171).

publication. Kurajew[1] showed that the vegetable ferment papayotin possessed the same power of producing precipitates in albumose solutions. Later, another Russian observer, Sawjalow,[2] gave a fairly complete account of the various chemical properties of this body. Lawrow and Salaskin,[3] however, conceive this formation not to be a true regenerative process, nor a real example of synthetic action, and, further, hold that it is the result of digestion under what might be termed unfavourable conditions. Again, Herzog[4] and Volhard[5] believe that this so-called plasteïn formation is not the result of the action of rennin at all, but may be regarded as another example of the reversibility of reaction of proteolytic ferments (Herzog), or of pepsin (Volhard). As to whether it is due to pepsin or rennin does not seem to be of very great importance, especially as some of the more modern workers like Pawlow and Parastschuk[6] hold that the so-called peptic action and the so-called rennet action are merely two phases of action of one and the same ferment.

Another theory of resynthesis was brought forward many years ago by Ott,[7] Popoff,[8] and Brinck,[9] who held that albumoses and peptones injected into the stomach of a living animal, or of one just dead, are converted into protein bodies in the stomach wall. It was further asserted that the protein which was formed was serum albumen. Brinck put this action down as being brought about by a micro-organism, to which she gave the name of *Micrococcus restituens.*

Hofmeister,[10] as I have already mentioned, held that on absorption the peptone was taken up by the leucocytes, and then, either through their agency or through that of the adenoid tissue, it is converted into cell protoplasm. Heidenhain denied this hypothesis, but both he and Shore[11] held to

[1] Kurajew, *Hofmeister's Archiv*, 1, 1901, 121.
[2] Sawjalow, *Pflüger's Archiv*, 85, 1901, 171.
[3] Lawrow and Salaskin, *Zeit. f. physiol. Chem.* 36, 1902, 277.
[4] Herzog, *Zeit. f. physiol. Chem*, 39, 1903, 305.
[5] Volhard, *Munch. med. Wochsch.* 50, 1903, 2129.
[6] Pawlow and Parastschuk, *Zeit. f. physiol. Chem.* 42, 1904, 415.
[7] Ott, *Archiv f. Physiolog.* 1, 1883.
[8] Popoff, *Zeit. f. Biol.* 25, 1889, 427.
[9] Brinck, *Zeit. f. Biol.* 25, 1889, 453.
[10] Hofmeister, *Schmiedeberg's Archiv*, 22, 1887, 306.
[11] Shore *Journ. of Physiology*, 11, 1890, 528.

the opinion that there was a change from peptone into albumen, but instead of the adenoid tissue they accredited the reaction to the epithelial cells of the small intestine. On the other hand, as regards Hofmeister's statements as to the part the leucocytes played in the various assimilative processes, a good deal of work has been done. Pohl[1] showed that during digestion there was a very marked leucocytosis, and, further, he stated that there was an excess of leucocytes in the mesenteric veins over the corresponding mesenteric arteries during digestion of proteids. This latter statement was controverted by Paton, Goodall, and Gulland,[2] who stated that no difference could be detected between the two. Erdély[3] recently confirmed Hofmeister's statement that the intestinal wall is richer in leucocytes when the animal has been well fed than when a fasting animal is examined. Erdély even goes so far as to suggest that the nature of the leucocytosis varies with the changes in diet. Paton and his co-workers have also demonstrated that there is a true post-prandial leucocytosis in which the lymphocytes showed a marked percentage increase. There is also some increase in the polymorphs, but there is practically no change in the number of the eosinophiles. They differ, however, from Hofmeister as regards the site of origin of the leucocytes: instead of arising, as Hofmeister said, from the intestinal lymphatic tissue, Paton and Goodall[4] proved that the white cells came from the bone marrow.

[1] Pohl, *Schmiedeberg's Archiv*, 25, 1889, 31.
[2] Goodall, Gulland, and Paton, *Journ. of Physiology*, 30, 1903, 1.
[3] Erdély, *Zeit. f. Biol.* 46, 1905, 119.
[4] Goodall and Paton, *Journ. of Physiology*, 33, 1905, 20.

THE WORK OF EMIL FISCHER AND HIS SCHOOL ON THE CHEMISTRY OF THE PROTEINS

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The Proximate Constituents

The remarkable researches of Emil Fischer upon the chemistry of the proteins during the last few years, which are of no less magnitude than his classical work upon the Carbohydrates and the Purine Compounds, are of such great importance that it seems desirable to give a somewhat detailed account of these investigations and to indicate the methods by which Fischer has arrived at his results.

Commencing with a minute study of the amino acids, which result in such variety as the products of hydrolysis of the proteins, Fischer next turned his attention to their separation as they result by hydrolysis, whereby a new method was perfected, which is now known as the ester method; and finally he has made extraordinary progress in the most important stage of synthesis.

Fischer has lately published the results of his work from 1899 to 1906 in book form, the opening pages of which consist of a lecture delivered to the Deutsche Chemische Gesellschaft in Berlin in January 1906. His researches are divided into three parts:

I. The Amino Acids.

II. The Hydrolysis of the Proteins.

III. The Polypeptides.

It has been known for many years from the work of Kühne, Neumeister, Chittenden, Schulze, Hlasiwetz, Habermann and others that the amino acids are the keystones of the protein molecule. It will make it easier to follow Fischer's subsequent work if we here give a list of the amino acids which have been

obtained up to the present time, as also their constitution in so far as it is known.

(a) *Monoaminomonocarboxylic acids:*

Glycine or amino-acetic acid.
Alanine or *a*-aminopropionic acid.
Valine or *a*-aminoisovalerianic acid.
Leucine or *a*-aminoisocaproic acid.
Isoleucine or *a*-aminomethylethylpropionic acid.
Phenylalanine or phenyl-*a*-aminopropionic acid.
Tyrosine or *p*-oxy-phenyl-*a*-aminopropionic acid } oxyamino acids.
Serine or *β*-oxy-*a*-aminopropionic acid } oxyamino acids.
Cysteine or *β*-thio-*a*-aminopropionic acid } thioamino acids.
Cystine or dicysteine, *a*-diamino-*β*-dithio-propionic acid. } thioamino acids.

(b) *Monoaminodicarboxylic acids:*

Aspartic acid or aminosuccinic acid.
Glutamic acid or *a*-aminoglutaric acid.

(c) *Diaminomonocarboxylic acids* (*hexone bases*)*:*

Ornithine or *a*-δ-diaminovalerianic acid.
Lysine or *a*-ϵ-diaminocaproic acid.
Arginine or *a*-amino-δ-guanidine valerianic acid.
Histidine or *β*-imidazoleaminopropionic acid.

(d) *Diaminooxymonocarboxylic acids:*

Diaminotrioxydodecanic acid.

(e) *Heterocyclic compounds:*

Proline or *a*-pyrrolidine carboxylic acid.
Oxyproline or oxy-*a*-pyrrolidine carboxylic acid.
Tryptophane or indoleaminopropionic acid.

This list, appalling as it looks, is in reality comparatively simple, and is evidence also as to the great complexity of the proteins, which consist of a combination of these amino acids in various proportions.

I. The Amino Acids

The amino acids as they are obtained from the proteins are, with the exception of glycine, in the optically active form. The synthetical products, on the other hand, are inactive or racemic,

i.e. consist of a mixture of equal parts of the dextro- and lævo-rotatory varieties. The real synthesis of the natural compounds is, therefore, only attained when the synthetical substances have been separated into their two constituents. Two methods may be used for this purpose: the biological method—destroying the one isomer by means of micro-organisms, such as *Penicillium glaucum* and *Aspergillus niger*—had been used by Schulze and Bosshard to obtain leucine and glutamic acid, and by Engel to obtain aspartic acid; but the chemical method of combining with optically active compounds to form salts and fractionally crystallising these salts had not been employed with any success. This was probably due to the small affinity which the simple monoamino acids have for acids and bases. The aminodicarboxylic acids, on the other hand, are moderately strong acids, which combine with bases to form salts of this kind, but the desired results were not achieved in the attempts thus made to separate the optically active forms.

Fischer found that by preparing the benzoyl derivatives of the amino acids, their acidic character was greatly increased and that they then formed with the optically active bases—strychnine, brucine, and morphine—stable salts which were soluble with greater difficulty than the amino acids and more easily isolated. Hippuric acid, or benzoylglycine, has been known for a long time; the derivatives of the other amino acids are similar in constitution. The ordinary process of benzoylating by the Schotten-Baumann method in the presence of excess of caustic soda gave poor yields of the benzoyl derivatives of the amino acids, but by substituting sodium bicarbonate for the caustic soda and using a large excess of benzoylchloride Fischer obtained excellent results, and succeeded in preparing the optical isomers of alanine, leucine, tyrosine, phenylalanine, aspartic and glutamic acids, as also of *α*-aminobutyric acid and *α*-amino-*n*-caproic acid.

For the preparation of large quantities of optically active amino acids, such as is required in the synthesis of the proteins, the benzoyl method for the separation of the isomers was not found to be convenient on account of the fact that the reconversion into the amino acid was often carried out with difficulty, since it required a long time and large quantities of acid (100 times the quantity of 10 per cent. hydrochloric acid) to completely hydrolyse the compound. Fischer had therefore to

discover another method. This consisted in the preparation of the formyl derivative by heating the amino acid with anhydrous formic acid at 100° C.; the separation of the inactive compound into its optically active isomers was again effected by means of the brucine salts; and finally the hydrolysis of the formyl compound was performed by heating for one and a half hours with ten times its quantity of 10 per cent. hydrochloric acid. Large amounts of active leucine and valine have thus been obtained. The optically active components of the latter compound, *a*-aminoisovalerianic acid, were first prepared by this method.

Quite lately Fischer and Jacobs have separated serine into its two optical isomers; neither the benzoyl derivative nor the formyl derivative gave the desired result, but they were successful when they used the *p*-nitrobenzoyl compound. Still more recently, the optically active forms of isoserine and diaminopropionic acid have been prepared.

It might be here mentioned that the biological method of separating the optically active compounds of the amino acids has recently been carried out by F. Ehrlich, who employed yeast in his experiments instead of the usual moulds.

In order to obtain the optically active amino acids, the racemic compound must first be synthesised. Two methods are generally employed for this purpose:

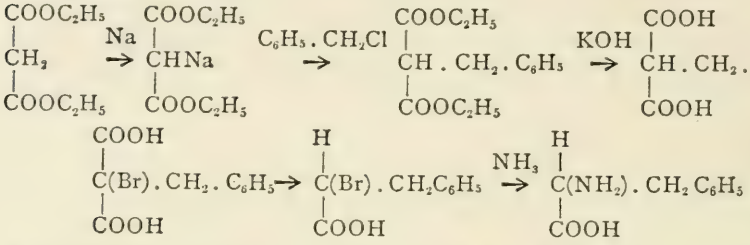
(1) The action of ammonia on the halogen fatty acid.

(2) The combination of aldehydes with hydrogen cyanide and ammonia and the subsequent hydrolysis of the amino-cyanohydrin.

Fischer has made use of both these methods: by the first method he prepared *a*-aminobutyric acid, *a*-amino-*n*-caproic acid, valine, and *a*-amino-*n*-valerianic acid. The latter two amino acids were also synthesised by the second or Strecker's method, by which isobutyl-*a*-aminoacetic acid or leucine was prepared from isovaleraldehyde. The identity of the benzoyl compound with that from natural leucine, as obtained from proteins and for a long time erroneously supposed to be *a*-amino-*n*-caproic acid, definitely proved the constitution of this amino acid, which had already been pointed out by E. Schulze and Likiernik in 1891, but had not been generally recognised.

Fischer has extended the first method by introducing a new mode of preparing the *a*-halogen fatty acid. This consists in

brominating the monoalkylmalonic acid, which, on heating, loses carbonic acid and is converted into the bromo fatty acid. Leucine can be readily prepared by this method from isobutylbromide and malonic ester; this method is especially useful for the synthesis of phenylalanine which is carried out by the following stages:



Isoleucine, which was discovered by F. Ehrlich in 1904 in beetroot residues, and later as a product of hydrolysis of proteins, is isomeric with leucine, but is not identical with the *a*-amino-*n*-caproic acid. Ehrlich considers it has the constitution of *a*-aminomethylethylpropionic acid $\begin{matrix}CH_3\\C_2H_5\end{matrix}\!\!>\!CH.CH.NH_2.COOH$ on account of its synthesis from *d*-amylalcohol, which was oxidised to *d*-valeraldehyde and then combined with hydrogen cyanide and ammonia, according to the second method of preparation; further, it gives *d*-amylalcohol on fermentation, whereas leucine gives isoamylalcohol. Bouveault and Locquin have also synthesised an *a*-aminomethylethylpropionic acid which appears to be identical with Ehrlich's isoleucine.

Tyrosine was not prepared by either of the above methods; benzoyltyrosine, as first synthesised by Erlenmeyer and Halsey by the reduction of *p*-oxy-*a*-benzoylaminocinnamic acid obtained from hippuric acid and *p*-oxybenzaldehyde, was employed by Fischer for the separation of the optically active isomers.

The second method was employed for the synthesis of the oxyamino acids. Serine, discovered by Cramer in 1864, is the simplest representative of this series, and was obtained by Fischer and Leuchs from glycollic aldehyde, hydrogen cyanide, and ammonia. Not only did this synthesis serve to definitely establish the constitution of serine, but it was the first instance of the employment of Strecker's method to build up oxyamino acids from oxyaldehydes. The constitution of serine was determined a little later by Erlenmeyer by a different method. It

was thus proved to be *a*-amino-*β*-oxypropionic acid, the isomeric compound, isoserine, being *β*-amino-*a*-oxypropionic acid.

Leuchs and Geiger have since found a better method for the synthesis of serine, from chloracetal, which is best described by the following formulæ:

$$Cl.CH_2.CH\begin{cases}OC_2H_5\\OC_2H_5\end{cases} \xrightarrow{NaOC_2H_5} C_2H_5O.CH_2.CH\begin{cases}OC_2H_5\\OC_2H_5\end{cases} \xrightarrow[\rightarrow]{\text{dil } H_2SO_4}$$

$$C_2H_5.O.CH_2.CHO \xrightarrow{HCN+NH_3}$$

$$C_2H_5.O.CH_2.CH(NH_2).CN \xrightarrow{HO} C_2H_5OCH_2.CH(NH_2).COOH \xrightarrow{\text{conc. HBr.}} HO.CH_2.CH(NH_2).COOH$$

By the same method Fischer and Leuchs have prepared *a*-amino-*γ*-oxyvalerianic acid, a compound which behaves like the other *γ*-oxy acids in easily forming an anhydride or *γ*-lactone giving the base

$$CH_3.CH-CH_2.CH(NH_2).CO$$
$$\quad\quad O_____|$$

This substance readily polymerises to a diketopiperazine, a condensation made much use of in the synthesis of proteins.

Like the oxyaldehydes, the carbohydrates combine with hydrogen cyanide and ammonia; thus, *d*-arabinose gives *d*-glucosaminic acid, which is identical with the product obtained by the oxidation of glucosamine. Fischer and Leuchs were by this means able to determine the constitution of this important compound.

The oxyamino acids, according to Fischer, are of extreme importance on account of their relationship on the one hand to the carbohydrates and on the other hand to the proteins, in which they probably occur to a much larger extent than is at present known.

Another oxyamino acid, *δ*-oxy-*a*-aminovalerianic acid, has been synthesised by Sörensen, by a method similar to that employed by Fischer in the synthesis of the diamino acids. It is isomeric with Fischer's *a*-amino-*γ*-oxyvalerianic acid, and constitutes another member of the series of the oxyamino acids.

Compared with the monoamino acids the diamino acids are much more difficult to obtain synthetically. The only diamino

acid prepared by synthesis before Fischer commenced his researches was diaminopropionic acid, which was obtained by Klebs in 1894 by the action of ammonia on dibrompropionic acid. The similar reaction with *a*-δ-dibromvalerianic acid did not lead to the synthesis of the expected diamino acid, but to the formation of *a*-pyrrolidine carboxylic acid (Willstätter, 1900).

By making use of Gabriel's phthalimide method for synthesis with a slight modification Fischer prepared *a*-δ-diaminovalerianic acid. The series of reactions occurring in the process are as follows:

$$C_6H_4\langle\begin{matrix}CO\\CO\end{matrix}\rangle NK + Br.CH_2.CH_2.CH_2Br + Na.CH\langle\begin{matrix}COOC_2H_5\\COOC_2H_5\end{matrix}$$
$$= NaBr + KBr + C_6H_4\langle\begin{matrix}CO\\CO\end{matrix}\rangle N.CH_2.CH_2.CH_2.CH\langle\begin{matrix}COOC_2H_5\\COOC_2H_5\end{matrix}$$

Bromination then gives

$$C_6H_4\langle\begin{matrix}CO\\CO\end{matrix}\rangle N.CH_2.CH_2.CH_2.C\begin{matrix}COOC_2H_5\\Br\\COOC_2H_5\end{matrix}$$

which, on hydrolysis and loss of carbon dioxide on heating, passes into phthalimidobromvalerianic acid:

$$C_6H_4\langle\begin{matrix}CO\\CO\end{matrix}\rangle N.CH_2.CH_2.CH_2.C\begin{matrix}H\\Br\\COOH\end{matrix}$$

This acid, on treatment with ammonia, whereby Br is exchanged for NH_2, and subsequent hydrolysis, yields *a*-δ-diaminovalerianic acid:

$$C_6H_4\langle\begin{matrix}COOH\\COOH\end{matrix} + H_2N.CH_2.CH_2.CH_2.CH(NH_2).COOH$$

The benzoyl derivative of this diamino acid only differed from Jaffe's ornithuric acid in optical activity; unfortunately, owing to lack of material, the separation of ornithine, as this diamino acid is called, into its stereoisomerides could not be carried out. Sörensen, however, some years later was able to accomplish this, and thus the synthesis of natural ornithine was completed. Ornithine is a product of the hydrolysis of arginine together with urea, and arginine was synthesised by Schulze and Winterstein in 1899 by the addition of cyanamide to ornithine.

By the above series of reactions Fischer prepared *a*-γ-diaminobutyric acid, and the same reactions would lead to the synthesis of lysine, which Fischer and Weigert obtained by a simpler and better method, consisting of the following reactions:

γ-cyanopropylmalonic ester, on treatment with nitrous acid, loses one of its carboxyethyl groups and forms the ethyl ester of α-oximido-δ-cyanovalerianic acid. On reduction with sodium amalgam this compound yields α-ϵ-diaminocaproic acid, thus:

$$NC.CH_2.CH_2.CH_2.CH\langle^{COOC_2H_5}_{COOC_2H_5} \rightarrow NC.CH_2.CH_2C(:N.OH).COOC_2H_5 \rightarrow$$
$$H_2N.CH_2.CH_2.CH_2.CH(NH_2).COOH$$

This acid has also been synthesised by Sörensen by a very similar method, but its separation into its stereoisomers has not yet been carried out and identity of one of its isomers with natural lysine confirmed.

Fischer and Schlotterbeck have prepared another diaminocaproic acid by heating sorbic acid with ammonia, but its structure has not yet been determined; another diaminovalerianic acid has been obtained from β-vinylacrylic acid.

The other hexone base, histidine, has not been synthesised by Fischer. Its constitution, as suggested by Pauly and proved by Windaus and Knoop, though not yet quite definitely determined, is that of imidazoleaminopropionic acid:

$$\begin{array}{l} NH - CH \\ | \quad \diagdown \\ CH = C \quad N \\ \qquad \diagup \\ \qquad -CH_2.CH.NH_2.COOH \end{array}$$

Most probably the amino group is in the α-position, but this still awaits the final proof.

Of the heterocyclic products, proline, as above mentioned, was prepared synthetically by Willstätter.

Two oxypyrrolidine carboxylic acids have recently been obtained by Leuchs in Fischer's laboratory, and deserve mention here in order to complete our knowledge of the amino acids. They were obtained as follows:

γ-chlor-β-oxypropylmalonic ester, prepared from epichlorhydrin and sodium malonic ester, loses alcohol and passes into the ester of δ-chlor-γ-valerolactone-α-carboxylic acid:

$$\begin{array}{l} \quad O \text{———} CO \\ \quad | \qquad\quad | \\ CH_2.Cl.CH.CH_2.CH.COOC_2H_5 \end{array}$$

This compound on bromination, hydrolysis with concentrated hydrobromic acid and subsequent splitting off of carbonic acid, gives α-brom-δ-chlor-γ-valerolactone, which on treatment

with ammonia gave two oxypyrrolidine carboxylic acids. One of these is probably the inactive form of the optically active oxyproline isolated by Fischer from the products of hydrolysis of gelatine.

Tryptophane was first isolated by Hopkins and Cole and supposed to be either scatoleaminoacetic acid or indoleaminopropionic acid. The work of Ellinger during the last few years points to its having the latter constitution.

The last amino acid which we have to consider is cystine. Our knowledge of this compound rests chiefly upon the work of Mörner and of Friedmann, who showed that on oxidation and loss of carbon dioxide it yielded taurine. Together with Suzuki, Fischer has prepared several of its derivatives and has compared the cystine as obtained from proteins on hydrolysis with that from cystine calculi. Contrary to the statement of Neuberg and Mayer, the two products were found to be identical, and Rothera's work in Cambridge was thus confirmed. At present, therefore, only the one cystine is a decomposition product of proteins, though the isomeric, isocystine, has been synthesised.

Finally, diaminotrioxydodecanic acid, which has only recently been isolated by Fischer and Abderhalden from the products of the hydrolysis of proteins, has not yet been synthesised, and its exact constitution is unknown.

Not only has Fischer synthesised the amino acids, but he has also prepared a large number of derivatives, in addition to the benzoyl and formyl compounds, whereby their isolation, purification, and recognition can be determined. The benzenesulpho derivatives, and especially the β-naphthalene sulpho derivatives, which are obtained by shaking an alkaline solution of the amino acid with the sulphochloride, are very useful for separating amino acids from very dilute or impure solutions. The latter derivative forms compounds with all the amino acids and with many of the polypeptides. It has been used by many workers to isolate amino acids from urine and other secretions (Samuely, Abderhalden and Schittenhelm, Lipstein, Emden and Reese).

The combination of the amino acids with phenylisocyanate in alkaline solution to form phenylureido acids, and the action of hydrochloric acid on these compounds to form their anhydrides, *i.e.* derivatives of hydantoine, first observed by Mouneyrat, has been much used to characterise the amino acids on account of the ease of crystallisation and sharp melting points

of these derivatives. With glycine the following reactions take place:

$$\begin{array}{l} CH_2.COOH \\ | \\ NH_2 \end{array} + C_6H_5.NCO = \begin{array}{l} CH_2.COOH \\ | \\ NH.CO.NH.C_6H_5 \end{array}$$

$$\begin{array}{l} CH_2.COOH \\ | \\ NH.CO.NH.C_6H_5 \end{array} = H_2O + \begin{array}{l} CH_2.CO \\ | \\ NH.CO \end{array} \!\!\rangle N.{}_6CH_5$$

They are similar in the cases of the other amino acids.

The most important of the derivatives of the amino acids are their esters, which are easily obtained by the action of gaseous hydrochloric acid and methyl or ethyl alcohol. These compounds were first prepared by Curtius, who has used them for his researches upon the aliphatic diazo compounds. As obtained on esterification, the esters are in the form of their hydrochlorides; from these Fischer obtained the free esters by the action of alkali at a low temperature and extraction with ether. The esters are liquids capable of distillation under diminished pressure, and have served as the means of the separation of the amino acids as they result on the hydrolysis of proteins. They have also been of great use in their synthesis.

Many attempts have been made to prepare the acid chlorides of the amino acids, and eventually it was found that they could be prepared by the combined action of phosphorus pentachloride and acetylchloride upon the amino acid. By their means the synthesis of the proteins was materially advanced, as all possible combinations of the amino acids together could be thus made.

II. The Hydrolysis of the Proteins

Of the various methods which have been employed in the study of the composition of the proteins, that of hydrolysis has been made use of most frequently, on account of the better and more certain results which are obtained thereby. The proteins can be hydrolysed by the action of acids, alkalies, and the proteoclastic enzymes; by either of these means a complex mixture of amino acids results. They are of two kinds—the monoamino and the diamino acids. To Drechsel, Hedin, and especially Kossel, we owe an excellent method for the separation of the diamino acids, which lends itself to a quantitative estimation of these compounds as they result on hydrolysis. The

separation and identification of the monoamino acids, on the other hand, by the older methods was extremely crude, and only where a large amount of one or two of the monoamino acids was present, was their isolation and characterisation carried out. Fischer, by introducing the ester method of separating the monoamino acids from one another, has advanced our knowledge of these acids considerably. His method, though not yet quantitative, gives us the means of obtaining about 70 per cent. of the total products.

In the majority of his experiments Fischer used concentrated hydrochloric acid as the hydrolysing agent, in some cases dilute sulphuric acid. This is particularly useful when tyrosine and diaminotrioxydodecanic acid only require isolation, the best results being obtained by boiling the protein for twelve to fifteen hours with five to six times its quantity of 25 per cent. sulphuric acid. This solution, after filtration and dilution with twice its volume of water, is neutralised with baryta, the excess of which is removed with sulphuric acid. The solution, after thoroughly washing out the precipitate of barium sulphate, is concentrated till these acids crystallise out. This method is extremely simple, but requires many days to carry out, as the filtration and washing out of the barium sulphate is very laborious. An improvement has recently been introduced by Abderhalden and Teruuchi, who use hydrochloric acid to hydrolyse the protein; after removal of the greater quantity of hydrochloric acid by evaporation in vacuo, the solution is treated with caustic soda in sufficient amount to exactly combine with the remainder of the hydrochloric acid, when the tyrosine crystallises out.

In all cases where the separation of tyrosine was not required, hydrochloric acid was used as the hydrolysing agent on account of its far greater convenience. The protein is covered with three times its quantity of concentrated hydrochloric acid in a flask; in a short time the majority of the proteins go into solution if the vessel be occasionally shaken, and the hydrolysis is completed by boiling the solution under a reflux condenser for five or six hours. The solution first becomes dark violet in colour and then dark brown; a large proportion of the hydrochloric acid escapes as gas, and a solution remains consisting of the hydrochlorides of the amino acids in 25 per cent. hydrochloric acid. Humin substances and fat-like masses, which separate

out, are filtered off after boiling with charcoal and the hydrochloric acid solution concentrated under reduced pressure. At this stage, if glutamic acid be present in considerable quantities, as in the case of caseinogen, it is separated as hydrochloride. The concentrated solution is therefore saturated with gaseous hydrochloric acid and allowed to stand in a cold place, when crystallisation occurs. The glutamic acid hydrochloride is filtered off, after mixing the solution with an equal volume of ice-cold alcohol; it is purified by dissolving in water, boiling with charcoal and again separating by saturating the solution with gaseous hydrochloric acid.

The next stage consists in the esterification of the amino acids. The acid solution is concentrated as far as possible in vacuo, the residue covered with absolute alcohol, and gaseous hydrochloric acid passed in, till the solution is saturated; it is finally warmed on the water bath. For every kilogram of protein three litres of absolute alcohol are required, as in the process of esterification a large amount of water results, which is harmful to the reaction. In order to complete the esterification the alcoholic solution is concentrated in vacuo to a thick syrup at a temperature not above 50° C., a fresh quantity of absolute alcohol is added, and the liquid again saturated with gaseous hydrochloric acid. This process may be repeated a second time. If glycine be present in the protein in considerable amounts, as in gelatine, it is isolated now as glycine ester hydrochloride. To effect this, a crystal of this compound is added to the solution, and it is allowed to stand for twelve hours at 0° C., when it crystallises out. It is filtered off whilst cold and washed with ice-cold alcohol, and is obtained pure after one recrystallisation from alcohol. A further separation of glycine ester hydrochloride occurs on concentrating the mother liquor, saturating with hydrochloric acid gas, and again allowing to stand. Almost all the glycine can be removed from the mixture in this way. The filtrate contains the ester hydrochlorides of the remaining amino acids, and it is concentrated in vacuo as much as possible at a temperature of 40° C. From this mass the esters are extracted by one of two methods.

The first and older method, which has been the most frequently employed, consists in adding about an equal volume of water and one and a half volumes of ether to the residue, thoroughly cooling in a freezing mixture, and adding strong

caustic soda solution until the free hydrochloric acid is neutralised, then an excess of finally granulated potassium carbonate. For convenience, the residue from a kilogram of protein is divided into four or six portions, which is of great importance in ensuring thorough cooling of the mixture. The esters of aspartic acid and glutamic acid, which are very sensitive to alkali, are thus first separated and dissolved by the ether which is poured off and renewed by adding a fresh quantity. The other esters are then liberated by adding 33 per cent. caustic soda, the whole mass being well shaken with the ether, which is from time to time renewed and replenished by another supply. Finally, sufficient alkali to combine with the whole of the hydrochloric acid and solid potassium carbonate is added, so that a pasty mass is formed, whereby the esters, which are very easily soluble in water, are salted out and extracted by the ether. Throughout the whole process thorough cooling is absolutely essential, and a large amount of ether is required.

The combined ethereal extracts are dried by shaking first with potassium carbonate for ten to fifteen minutes, from which they are poured off, and then for twelve hours with anhydrous sodium sulphate. The ether is distilled off, preferably at the ordinary temperature in vacuo, and a brown oil remains, consisting of the esters of the amino acids.

In the process of extraction a certain quantity of the esters is destroyed by the alkali and therefore not taken up by the ether. The amino acids can be recovered from this by neutralising with hydrochloric acid, filtering off the potassium chloride as it separates on concentration, extracting the residue with alcohol, and repeating the above process of esterification. This may be repeated again, but a certain loss still occurs. To avoid this as far as possible it is preferable to liberate the esters from their hydrochlorides by means of sodium ethylate. This is the second and newer method. The hydrochloric acid is therefore removed as completely as possible by evaporation in vacuo, the residue dissolved in five or six times its quantity of alcohol, and in a small portion of this the amount of chlorine is determined. The calculated amount of sodium dissolved in alcohol to combine with the whole of the chlorine is then added to the thoroughly cooled solution, the sodium chloride formed filtered off, and the alcoholic solution evaporated under greatly diminished pressure. A certain quantity of the lower-boiling

esters pass over into the distillate, but they can be recovered by acidifying with hydrochloric acid and evaporating, when they are obtained as amino acid hydrochlorides. The dark brown residue which remains in the distilling flask is fractionally distilled in vacuo, leaving a mass consisting of tyrosine and other amino acids, which in the first method are contained in the mass of potassium carbonate.

The distillation in vacuo is carried out first at the pressure of 10–12 mm. produced by the water pump, and then at a pressure of 0·5 mm. produced by a Geryck pump. At 10–12 mm., three fractions (up to 60°, 80°, 100°) are collected, the temperature taken being that of the water bath; the distillation is then continued at 0·5 mm., the first fraction being that at the temperature of the boiling water bath; several fractions are then collected up to 160°, the temperature being procured by the use of an oil bath. The residue, which does not distil at this temperature, consists of a dark brown oil; it sets to a hard mass on cooling, and contains, amongst other substances, leucinimide.

The various fractions, so obtained, consist of a mixture of the esters of two or more amino acids, and they are again fractionated in vacuo; this is not essential for the fractions distilling above 100° C. at 0·5 mm. pressure, which contain the esters of phenylalanine, aspartic acid, glutamic acid, and serine. These esters are separated by taking advantage of their different solubilities in water, ether, petroleum ether; *e.g.* phenylalanine ester is soluble with difficulty in water, and is extracted from the aqueous solution of the esters with ether, the esters of aspartic and glutamic acids remaining in the water. Serine ester is insoluble in petroleum ether; on mixing the fraction containing it with petroleum ether it separates as an oil, the esters of the three other acids remaining in solution.

Finally the esters must be converted into the amino acids; in the case of the lower-boiling fractions, they are hydrolysed by boiling with water under a reflux condenser for five to six hours until the alkaline reaction disappears; leucine may crystallise out during this process. The esters in the higher-boiling fractions which contain glutamic and aspartic acids are hydrolysed by boiling with baryta; if hydrolysed by water, they remain as acid esters. On cooling, aspartic acid may separate as the barium salt of the racemised acid. The

phenylalanine ester fraction is hydrolysed by evaporating with concentrated hydrochloric acid, when the hydrochloride is obtained.

The ester method can be combined with the methods for obtaining tyrosine and the diamino acids. The protein is therefore hydrolysed by sulphuric acid, the tyrosine separated by crystallisation, and the diamino acids precipitated by phosphotungstic acid. The filtrate, after removal of phosphotungstic acid, is treated as above described for the separation of the monoamino acids.

From the above it is obvious that a mixture of amino acids is still present; their final separation, as they result from the various fractions, has still to be carried out. The methods employed are best described by taking a particular protein as illustration, *e.g.* crystallised oxyhæmoglobin, which was hydrolysed by Abderhalden by the above method, and gave five fractions of esters from which the appended weights of amino acids were isolated:

Temperature.	Pressure.	Weight of Ester.	Weights of Amino Acids.
Fraction I. To 40° (vapour)	10 mm.	64·2 gm.	14·2 gm. alanine.
„ II. 40—60° „	10 „	107·0 „	26·0 gm. alanine, 30·7 leucine, 1 gm. protein.
„ III. 100° (water bath)	0·2 „	403·4 „	247·5 gm. leucine, 21.5 gm. protein.
„ IV. 100—130° (oil bath)	0·2 „	81·7 „	19·5 gm. phenylalanine, 11·3 gm. aspartic acid, 4·2 gm. glutamic acid, 1·7 gm. serine.
„ V. 130—160° „	0·2 „	93·0 „	21·1 gm. phenylalanine, 4·1 gm. glutamic acid, 11·0 gm. aspartic acid, 1·0 gm. serine.

Oxyhæmoglobin contains no glycine, so that its separation was not necessary, but as ester hydrochloride four-fifths can be isolated when the protein contains 20 per cent. in its molecule; the remainder is distilled as ester, and passes into the first fraction with alanine ester, from which it is separated by conversion into its ester hydrochloride. The alanine is then separated by crystallisation, after removal of the hydrochloric acid, and is characterised by conversion into its benzoyl derivative. Owing to partial racemisation occurring during the

processes of hydrolysis and esterification, its rotation is generally found too low, and the melting-point of its benzoyl derivative is not quite sharp.

The presence of valine, or α-aminoisovalerianic acid, is not mentioned in this paper. Its separation from leucine, with which it is generally present, is carried out with difficulty, and depends on the fractional crystallisation of the free acids and their copper salts.

Leucine, again, as it results on hydrolysis, is partially racemised. Its characterisation is therefore more difficult, but this is carried out by completing the racemisation by heating it with baryta at 170–175° C. for twenty-four hours, and then converting it into its hydantoin derivative. Isoleucine is probably mixed with leucine, but no special separation of this isomer was performed by Fischer.

Proline is the only amino acid which is easily soluble in absolute alcohol. By evaporating the mixture in which it is contained to dryness and extracting with absolute alcohol, it can be easily isolated. This compound, when obtained by hydrolysis, also exists in the racemic and optically active forms; by conversion into the copper salt the two varieties can be separated by alcohol in which the copper salt of the active form is soluble. The racemic acid is characterised by a determination of the amount of water and the content in copper of its copper salt, the active acid by its hydantoin derivative.

Phenylalanine is so easily isolated as ester that an analysis of its hydrochloride, obtained by evaporating the ester to dryness with hydrochloric acid, is sufficient for its identification. Small quantities can be identified by oxidation to phenylacetaldehyde, which has a very distinctive smell.

Serine, on account of the insolubility of its ester in petroleum ether, generally requires no great amount of work to characterise it. After hydrolysis by baryta, the solution is concentrated when it crystallises out, and elementary analysis determines its nature. The β-naphthalene sulpho-compound may be used, if further characterisation be necessary.

Aspartic and glutamic acids are separated partially after the hydrolysis of their esters by baryta, when the barium salt of dl-aspartic acid crystallises out on cooling. A further quantity may be isolated by crystallisation of the free acid, but the

remainder is only obtained after the removal of the glutamic acid as hydrochloride.

The ester method has not led to the discovery of a large number of new amino acids; to this category proline and oxyproline belong. The possibility that proline is a secondary product formed by the action of acid on another amino acid is negatived by the fact that it results when the proteins are hydrolysed by alkali. The great use of the ester method has been to separate the amino acids, and to determine their great variety in all the proteins; formerly, only those existing in large amounts, such as tyrosine, leucine, aspartic acid, were isolated. The ester method also gives larger amounts. Alanine, serine, and phenylalanine, which was only once isolated by Schulze from plant proteins, were seldom found, but now their universal presence in all proteins is definitely established. Phenylalanine, in its distribution, is the chief aromatic compound, since tyrosine does not occur in all proteins, and it often exceeds in amount that of tyrosine when both exist in the same protein.

These results can be seen at a glance by referring to the following table, which also shows the various amounts of individual amino acids in percentages, as they result on the hydrolysis of proteins:

	Globin from oxyhæmoglobin.	Caseinogen.	Gelatine.	Silk fibrin.	Egg-albumin.
Glycine	...	...	16·5	36·0	...
Alanine	4·2	0·9	0·8	21·0	2·1
Valine	present	1·0	1·0	...	...
Leucine	29·0	10·5	2·1	1·5	6·1
Proline	2·3	3·1	5·2	present	2·2
Phenylalanine	4·2	3·2	0·4	1·5	4·4
Glutamic acid	1·7	11·0	0·9	...	8·0
Aspartic acid	4·4	1·2	0·6	present	1·5
Cystine	0·3	0·06	...	...	0·2
Serine	0·6	0·23	0·4	1·6	...
Tyrosine	1·5	4·5	...	10·5	1·1
Lysine	4·3	5·8	2·7	present	...
Arginine	5·4	4·8	7·6	1·0	...
Histidine	11·0	2·6	0·4	present	...
Oxyproline	...	...	3·0	...	...
Tryptophane	...	...	...	...	present

Not only has Fischer hydrolysed proteins by acids and by alkali, but also by the enzymes pepsin and trypsin. The latter gives essentially the same products as acids, but its action is

not complete. Even on prolonged action, when the biuret reaction is absent or only very faint, a complex product together with amino acids results. This complex polypeptide, as it is termed, contains the whole of the proline and phenylalanine contained in the protein, together with other amino acids. It is apparently not identical with Kühne's antipeptone which gave the biuret reaction. On complete hydrolysis by acid, it yielded the same quantity of proline and phenylalanine as would result from the protein by acid hydrolysis.

In the animal body the action of trypsin is preceded by that of pepsin. If a protein be first digested by pepsin and then by trypsin, the same complex polypeptide might be expected to result, but this was not the case. It was less in amount, and did not contain the whole amount of the proline and phenylalanine of the protein, a certain portion of these being found with the other amino acids, which had been split off. It would thus appear as if the protein molecule contained a special nucleus, as suggested by Kossel, who believes that it consists mainly of the diamino acids; these make up 90 per cent. of the protamine molecule, the protein molecule containing less of this nucleus and more monoamino acids than the protamine. Histones are a transition product between the protein and protamine. Unfortunately, by the method adopted by Fischer, a nucleus consisting mainly of diamino acids could not be definitely determined, since the phosphotungstic acid used not only precipitated the complex polypeptide, but also the diamino acids, so that it was impossible to tell whether they were free or combined together in this polypeptide. Subsequent work, if attended with such far-reaching results, will certainly decide this question, especially as less complex combinations of amino acids have already been isolated from proteins. New amino acids and oxyamino acids may still be discovered; Skraup's results point to the existence of two more diamino acids and other oxy-amino acids; their exact constitution is still very uncertain. His work cannot be further mentioned here.

The hydrolysis of various proteins has been carried out by Abderhalden and several co-workers in the hope of being able to obtain closer relationships between the members of the various classes of proteins, and more distinctions between the classes. Abderhalden's book on physiological chemistry brings out these points as well as many others of great interest.

III. The Synthesis of the Proteins. The Polypeptides

Many theories have been advanced concerning the constitution of the proteins as to how the amino acids, the key-stones, are combined together. The majority of the views regard the amino acids as being bound together in an anhydride-like manner in the form of acid amides. This view has been very strongly supported by Hofmeister, and several experimenters had already attempted to combine the amino acids together in this manner. Thus, Schaal made the anhydride of aspartic acid, which was further condensed by Grimaux and Schiff. Schutzenberger condensed amino acids with urea, Lilienfeld employed a mixture of amino acid esters, and Balbiano and Frasciatta obtained a horny mass, an anhydride of glycine.

Not, however, until Fischer commenced his systematic researches upon the combination of the amino acids with one another in 1901 was any real progress made. Since then the enormous number and variety of products which have been prepared by Fischer and his pupils have given us an actual knowledge as to how the proteins are built up, and they show us how much more complicated the proteins are than the fats and carbohydrates, the other natural constituents of animal and vegetable cells. The combinations of the amino acids have been called by Fischer polypeptides, those containing two, three, four, etc., acids being di-, tri-, tetra-peptides, etc. They remind us in their nomenclature of monosaccharides, dissacharides, polysaccharides, and of peptone, which Fischer regards as a mixture of polypeptides.

It was found by Curtius and Goebel many years ago that the ethyl ester of glycine, when dissolved in water, lost two molecules of alcohol, and was converted into diketopiperazine, or, as it is now better called, glycine anhydride,

$$\begin{array}{l} NH\,.\,CH_2\,.\,CO \\ \quad\quad\quad\

This compound is glycylglycine, as termed by Fischer, the group $NH_2.CH_2.CO$ being called the glycyl group. It might be mentioned that the benzoyl derivative of this compound was obtained by Curtius in 1882.

The ester of this compound was formed when the glycine anhydride was boiled with alcoholic hydrochloric acid. Both free acid and the ester have a great tendency to change back into the anhydride, a property which has been utilised in isolating such compounds from the products of hydrolysis of proteins. The amino group is also very reactive, *e.g.* with chlorcarbonic acid ester it gives carbethoxyglycylglycinester, from which, on hydrolysis, glycylglycinecarboxylic acid is obtained. When heated with leucinester, carbethoxyglycylglycinester undergoes a similar condensation, forming carbethoxyglycylglycylleucine, where three molecules of amino acids are coupled together.

The diketopiperazine of alanine, and that of leucine, the so-called leucinimide, both gave the corresponding dipeptides alanylalanine and leucylleucine,

$$H_2N.CH(C_4H_9).CO.NH.CH(C_4H_9).COOH.$$

Difficulties were, however, experienced with the condensation of the other amino acid esters, and with the more complex esters of the dipeptides, as also with the condensation of dissimilar amino acids, where several products may result, so that progress by these means became almost impossible. The compounds so prepared, as expected from their constitution, gave the red biuret reaction, which was shown by Schiff to be given by compounds having $CO-NH$ groups joined together directly, or by a C atom or by a N atom.

About this time (1902) Curtius and Benrath prepared benzoylpentaglycinaminoacetic acid, and two years later Curtius showed that his biuret base was triglycylglycinester. This compound is the ester of Fischer's triglycylglycine. Triglycylglycinester, according to Curtius and Gumlich, changes into an anhydride, which is an octapeptide.

The method of preparing dipeptides from the diketopiperazines a few years later again proved useful, when it was found that by heating the esters of the amino acids to 150–180° combination occurred, and instead of splitting open the ring by boiling with concentrated hydrochloric acid, dilute alkali at the ordinary

temperature produced the same result in fifteen to twenty minutes. The diketopiperazines of alanine, leucine, phenylalanine, tyrosine are thus easily prepared, as also the corresponding dipeptide. As yet the dipeptides of the diamino acids, lysyllysine and histidylhistidine and serylserine, have only been obtained by this method by Fischer and Suzuki; in these cases the methyl ester of the amino acid was more easily converted into the diketopiperazine. The method does not lend itself to the preparation of mixed diketopiperazines. These are obtained by the action of ammonia on the *a*-halogenacyl derivative of the amino acid ester, *e.g.* glycylalanine anhydride,

$$Cl.CH_2.CO.NH.CH(CH_3).COOC_2H_5 + 2NH_3 = NH_4Cl + C_2H_5OH +$$
$$NH\begin{cases} CH_2.CO \\ CO.CH.CH_3 \end{cases}NH$$

The simple method of combining the acid chloride of an amino acid with another amino acid for the synthesis of polypeptides could not be employed, as these derivatives were unknown and impossible to prepare. The carbethoxy derivative, however, of the amino acid, or polypeptide, when warmed with thionylchloride, was found to give the acid chloride, and this compound reacted with the esters of amino acids or polypeptides to form the carbethoxy derivates of polypeptides, thus carbethoxyglycylglycine on treatment with thionylchloride yielded the acid chloride which reacted with glycylglycinester to form the carbethoxy derivative of triglycylglycinester. The conversion of this compound into the true tetrapeptide, owing to the firm union of the COOH group, was not possible, and a third method, which reminds us of the preparation of the amino acids, was attempted, and proved most fruitful.

This method consists in introducing an *a*-halogenacyl residue into the molecule of an amino acid or polypeptide, and then treating this compound with ammonia, whereby the Cl is exchanged for NH_2; thus chloracetyl tyrosine, obtained from chloracetyl chloride and tyrosine, on treatment with ammonia gives glycyltyrosine,

$$CH_2Cl.COCl + HOOC.CH(NH_2).CH_2.C_6H_4OH = HCl +$$
$$HOOC.CH(NH.CO.CH_2Cl).CH_2.C_6H_4OH$$
$$.HOOC.CH(NH.CO.CH_2Cl).CH_2.C_6H_4OH + NH_3 = HCl +$$
$$HOOC.CH(NH.CO.CH_2.NH_2).CH_2.C_6H_4OH$$

and chloracetylglycylglycinester gives diglycylglycinester, from which on hydrolysis the free acid is obtained.

In just the same way the alanyl and leucyl radicles can be introduced by using *a*-brompropionylbromide and *a*-bromisocapronylchloride. Leucylproline can be obtained in this manner, the *a*-pyrrolidine carboxylic acid also lending itself to synthesis by this reaction. The residue—prolyl—can be introduced by combining amino acids with the chloride of *a*-δ-dibromvalerianic acid; the compound obtained on treatment with ammonia, just as in the synthesis of *a*-pyrrolidine carboxylic acid, after exchanging the Br atoms for NH_2, loses ammonia, forming the prolyl dipeptide, *e.g.* prolylalanine. Not only can the simple monoamino acids be combined by this method, but also cystine, from which, *e.g.*, dialanylcystine results. The aspartic acid radicle is introduced by means of fumarylchloride; when combined with alanine, fumaryldialanine is formed; this adds on ammonia, yielding asparagyldialanine. Lastly, the phenylalanine residue can be attached by means of phenylbrompropionyl chloride.

A large number of mixed dipeptides, and also of tri-, tetra-, etc., peptides, have been prepared by this method, which, however, only admits of the lengthening of the chain in one direction —namely, on the side of the amino group.

It was of extreme importance to be able to lengthen the chain upon the side of the carboxyl group, and this is possible only by means of the acid chloride. As previously stated, the acid chlorides of the amino acids and polypeptides were not known, but that the acid chloride of the carbethoxy derivative was prepared by the action of thionylchloride upon it. The presence of the halogenacyl radicle in the amino acid, such as bromcapronyl, again allows of the formation of the acid chloride of the amino acid or polypeptide, and this compound reacts with the esters of other amino acids in the following way:

$$C_4H_9 . CHBr . CO . NH . CH_2 . COCl + 2NH_2 . CH_2 . COOC_2H_5$$
$$= HCl . NH_2 . CH_2 . COOC_2H_5 +$$
$$C_4H_9 . CHBr . CO . NH . CH_2 . CO . NH . CH_2 . COOC_2H_5$$

Subsequent hydrolysis of the ester and treatment with ammonia gives the polypeptide. This process can be carried out indefinitely by again making the acid chloride of the new compound and condensing it with an amino acid or a polypeptide. Any conceivable combination can be prepared by

this method, *e.g.* leucylalanine, alanylleucine, alanylleucylglycine, leucylalanylglycine, glycylalanylleucine, etc.

Fischer has ultimately been able to prepare the acid chloride of the amino acid so that it can be used directly for synthesis. Whereas the acid chlorides of the halogenacyl derivatives were easily prepared by the action of phosphorus chloride, this did not take place with hippuric acid. After many attempts to prepare hippuryl chloride, success at length was attained when hippuric acid was heated with acetylchloride and phosphorus pentachloride together. By simply extending this method of preparation to the amino acids themselves, *i.e.* by shaking them in a finely powdered condition with acetylchloride and phosphorus pentachloride, Fischer obtained the acid chlorides of the amino acids, whereby a step in the synthetical processes can be saved.

During the course of the last year several higher polypeptides have been synthesised by these methods; these are two hexapeptides, a heptapeptide, an octapeptide, and even a dodekapeptide. They all consist principally of glycine residues, the dodekapeptide having one leucine and eleven glycine radicles. Such long glycine chains probably do not exist in the protein molecule, except possibly in gelatine, which contains about 20 per cent. glycine. Their study, however, is of great importance in that they cannot exist in stereoisomeric forms, and the knowledge of their properties may lead to the isolation of a higher polypeptide from the proteins themselves. Further, the material is of easy access, glycine being readily prepared synthetically, which is not the case with the other amino acids, many of which are more easily obtained by the hydrolysis of proteins than by synthesis.

In a quite recent paper Fischer gives an account of the preparation of an octadecapeptide, built up of fifteen glycine and three l-leucine residues, *i.e.* a polypeptide containing six more members in its molecule than the above-mentioned dodekapeptide. Its preparation is of great interest, as it shows very clearly how the synthetical method has been employed in these researches. Pentaglycylglycine and d-*a*-bromisocapronyldiglycylglycine, $BrCH(C_4H_9)CO.[NHCH_2CO]_2.NHCH_2COOH$ are used. The acid chloride of the latter compound is first prepared and combined with the hexapeptide. The bromo compound so obtained on treatment with liquid ammonia gives l-leucyl-

octaglycylglycine. This decapeptide, on combination with bromisocapronyl diglycylglycine, and subsequent replacement of Br by NH_2, yields the tetradecapeptide

$$NH_2CH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_5.NHCH_2COOH,$$

and a repetition of the process of combining with bromisocapronyldiglycylglycine gives the octadecapeptide

$$NH_2CH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_3.NHCH(C_4H_9)CO.[NHCH_2CO]_5.NHCH_2COOH.$$

Many technical difficulties had to be surmounted in its preparation which were successfully overcome; *e.g.* prevention of frothing by shaking up with glass beads, etc.

The molecular weight of this compound is 1213. It far exceeds that of the fats, of which tristearin has the molecular weight 891, and it belongs to the most complicated combinations ever obtained synthetically without our losing the knowledge of its constitution. If, in place of the glycine residues, it contained tyrosine, phenylalanine, or leucine residues, its molecular weight would be increased two- to three-fold, *i.e.* to values taken as the normal for many natural proteins. The values of 12,000—15,000 for other proteins, according to Fischer, are uncertain, as we have no guarantee of the purity of these natural substances.

Up to the present time nearly one hundred different polypeptides have been prepared. Their structure is derived directly from their synthesis, and they are generally represented as in the formulæ given in the above equations. Other structures are also possible; indications of these have been observed in their study, and they are at present only of theoretical interest.

The configuration of the polypeptides is of extreme importance, for, with the exception of glycine, all the amino acids contain an asymmetric carbon atom. According to van't Hoff's formula, 2^n, the number of isomers possible when two amino acids are combined together is four, namely, the dd, ll, dl, ld forms; and two racemic forms—dd, ll and dl, ld—can be obtained from these. With more amino acids coupled together the complications are still greater, and their separation well-nigh impossible. Fortunately, these conditions do not seem to be present in the protein molecule, since the amino acids as they result on hydrolysis are optically active andl hence exist in

only one form. Optically active polypeptides, in addition to glycyl-l-tyrosine and leucyl-l-tyrosine, have quite recently been prepared by Fischer. These are l-leucylglycine, l-leucyl-d-alanine, l-leucyl-l-leucine, and they are composed of the stereoisomers obtained from the proteins. Glycyl-d-alanine, d-alanyl-d-alanine, and d-alanyl-l-leucine have since been synthesised. Their synthesis is far more tedious, since it necessitates the preparation of the optically active forms. In their preparation the acid chloride method has been used, and a curious change from the dextro to the lævo form, termed Walden's rearrangement, has been observed by Fischer, thus d-leucine is converted into isobromcaproic acid chloride; this when combined with l-leucine and treated with ammonia gives l-leucyl-l-leucine, instead of the expected d-leucyl-l-leucine.

The material employed in the preparation of nearly all the polypeptides consisted of the racemic amino acids, from which two racemic compounds would be expected to result. These first appear in the halogen intermediate compounds,

$$\mathrm{Br.\mathbf{C}.HR.CO.NH.\mathbf{C}HR.COOH}$$[1]

and a separation in a few cases has been effected at this stage. In other cases both the dipeptides have been isolated after their formation. In the majority of cases, however, only one product has been obtained; this may either consist of mixed crystals of the two forms, or only the one form may have resulted. Instances of this kind have been noticed in other classes of compounds, and it can only be assumed that in the synthesis one arrangement is more favourable than the other, and therefore appears in much larger amount under the influence of asymmetry, or the velocity of the reaction between one pair is greater than between the other pair. The influence of asymmetry is well known to play a great part in the rate of reactions, as exemplified in the action of enzymes. Both forms may also be present, but the one in greater quantity than the other.

The nomenclature of the polypeptides in the case of those consisting of optically active amino acids is quite simple, *e.g.* d-leucyl-l-alanine, but where the racemic forms are concerned it is more difficult. The examples, dl-leucylglycine and glycyl-dl-leucine, designate the two isomeric inactive dipeptides, containing only one asymmetric carbon atom. The two racemic dipeptides

[1] The asymmetric carbon atoms are in thicker type.

with more than one asymmetric carbon atom are represented as follows:

d-alanyl-d-leucine l-alanyl-l-leucine	I.	d-alanyl-l-leucine l-alanyl-d-leucine	II.

but as their configuration must be first established, Fischer has simply labelled them A and B, the A denoting the more insoluble. The synthesis of the optically active forms and their combination together would definitely decide the constitution, but a simpler method has been found by studying the action of trypsin upon them. The A combination was hydrolysed; d-alanyl-l-leucine contains the natural amino acids, and hence the form II. most probably corresponds to the more insoluble racemic dipeptide.

The configuration of the diketopiperazines is far more complex and need not be entered into here.

Although the amino acids are in all probability joined together in the manner of acid amides, it is possible that other couplings may also be present in the protein molecule. Ethers of the oxyamino acids, their esters with other amino acids are conceivable; and polyoxyamino acids, if present, suggest a still more complex combination. The presence of diketopiperazine rings is also likely, as they are so easily converted by alkali into the dipeptides; a change of this kind may take place in the formation of the so-called albuminates and on coagulation. The hydrolysis of the polypeptides by the proteolytic enzymes, and their giving of the red biuret reaction like peptone, point most strongly to the acid amide manner of combination of the amino acids in the protein molecule.

IV. The Action of Enzymes upon the Polypeptides

We have seen that, by synthesis, a very large number of polypeptides have been prepared from the amino acids; their number is almost infinite, even if only the optically active forms, as they occur in nature, be coupled together. Some light might be shed upon what combinations actually occur in the protein molecule by the study of the products of hydrolysis, when this is not carried to the ultimate stage. Such intermediate products are already known and designated proteoses and peptones. They consist of mixtures of complex polypeptides, and at present our methods are not sufficiently perfect for separating polypeptides from one another and from amino acids. They

result when the proteins are hydrolysed by the proteolytic enzymes, pepsin, and trypsin. Even under identical conditions it is difficult to determine how far hydrolysis has proceeded under their influence, as two preparations are scarcely ever of the same strength. Hydrolysis by acids and alkalies is not so limited, as they tend to give the ultimate end products which at the same time undergo partial racemisation.

The study of the behaviour of the synthetical polypeptides to trypsin throws much more light upon the combinations which may actually occur in the protein molecule. Each protein may again possess different combinations; their properties, as also the various amounts of the same amino acid, obtained by hydrolysis, point to a variety of combinations.

Fischer commenced his investigations upon this subject in conjunction with Bergell at a time when only a few of the dipeptides had been prepared. The hydrolysis of these by trypsin was found to depend upon various factors. Further and much more complete investigations were carried out with Abderhalden, as the result of which the synthetical polypeptides could be divided into two classes:

Those Hydrolysed	*Those not Hydrolysed*
*alanylglycine	glycylalanine
*alanylalanine	glyclyglycine
*alanylleucine A	alanylleucine B
*leucylisoserine A	leucylalanine
glycyl-l-tyrosine	leucylglycine
leucyl-l-tyrosine	leucylleucine
*alanylglycylglycine	aminobutyrylglycine
*leucylglycylglycine	aminobutyryl-aminobutyric acid A
*glycylleucylalanine	aminobutyryl-aminobutyric acid B
*alanylleucylglycine	aminoisovaleryl-glycine
dialanylcystine	glycylphenylalanine
dileucylcystine	leucylproline
tetraglycylglycine	diglycylglycine
triglycylglycinester (Curtius' biuret base)	triglycylglycine
	dileucylglycylglycine

The two classes show firstly the endless variety of the simple polypeptides, and secondly, on closer examination, that

* These are racemic compounds.

their hydrolysis by trypsin depends upon several factors. The influence of their structure is exemplified by alanylglycine which is hydrolysed, the isomeric glycylalanine not being hydrolysed. Similarly, alanylleucine A is acted upon, but not leucylalanine. The influence of the individual amino acid is more marked; thus, where alanyl is the acyl radicle, hydrolysis takes place; but not when leucyl or aminoisovaleryl (valyl) are in this position. Hydrolysis also occurs when tyrosine, serine, and cystine stand at the end of the chain.

The configuration of the molecule is also of importance; the racemic compounds are hydrolysed asymmetrically with the formation of the optically active amino acid which occurs in the proteins; thus alanylleucylglycine gave d-alanine and leucylglycine mixed with the other form originally contained in the racemic tripeptide. The identification of the racemic form in the case of the combinations of alanine and leucine by means of trypsin has been previously mentioned. Alanylleucine A was partially hydrolysed, and was supposed to be composed of the naturally occurring amino acids. This has quite recently been proved by the study of the action of trypsin upon polypeptides built up entirely of optically active amino acids. Again, leucylleucine was not attacked, and it is now shown that it is the racemic compound, l-leucyl-d-leucine and d-leucyl-l-leucine.

The following list gives these recent results:

Those Hydrolysed	*Those not Hydrolysed*
d-alanyl-d-alanine	d-alanyl-l-alanine
d-alanyl-l-leucine	l-alanyl-d-alanine
l-leucyl-l-leucine	l-leucyl-glycine
l-leucyl-d-glutamic acid	l-leucyl-d-leucine
	d-leucyl-l-leucine.

The number of amino acids in the molecule, *i.e.* the length of the chain, is very interesting in the case of the glycine polypeptides. Not until five glycine radicles, as in tetraglycylglycine, are present, is hydrolysis produced by trypsin. It is curious to note that the ester of triglycylglycine is hydrolysed, but not the free acid. This is the biuret base of Curtius, and was previously examined by Schwarzschild in its behaviour to trypsin. It is probable that the configuration of the dileucyl group in dileucylglycylglycine prevents its hydrolysis by trypsin,

as leucylglycylglycine is acted upon, though not leucylglycine. None of these polypeptides was hydrolysed by pepsin. In all probability the chain is not sufficiently long, and it would be interesting to know the result of the action of pepsin upon the recently prepared octapeptide and dodekapeptide.

The results undoubtedly show which combinations are most likely to occur in the protein, and they are of great importance for their synthesis, as only those polypeptides containing optically active amino acids combined together in the way that trypsin acts upon them need be prepared.

In the earlier experiments, an extract of dried pancreas was employed as the hydrolytic agent, but in the later experiments pure pancreatic juice, obtained from a pancreatic fistula by Pawlow, was used. There was a striking difference in their behaviour to certain dipeptides—namely, leucylalanine, leucylglycine, leucylleucine. These were not hydrolysed by the pure juice, but by the extract of dried pancreas, which would contain the autolytic enzyme as well as the enzyme trypsin, they were hydrolysed.

These differences of enzymes to polypeptides may show differences in the pancreatic juice of various animals; it is well known that some animals are able to digest substances left untouched by others, and further, that though not hydrolysed by trypsin, they may be attacked and converted into amino acids by the enzymes of other organs such as the liver, spleen, etc., and thus made assimilable by the animal organism.

Investigations as to whether the various polypeptides are hydrolysed by the organism have been made by Abderhalden and several co-workers, by injecting them and ascertaining whether they were excreted as such or as their constituent amino acids in the urine. These products were not found in the urine, and it is concluded that they are utilised in the same way by the organism as the proteins themselves are in metabolism, *i.e.* they are split up and their nitrogen excreted as urea. In fact, not only were the optically active compounds utilised, but also the racemic compounds. Slight differences were noticed between dogs and rabbits which did not entirely hydrolyse the racemic products, *e.g.* dl-leucylglycine. The anhydride of leucylglycine and glycocoll were found in the urine when somewhat large quantities were injected.

The synthetical polypeptides have also been employed by

Abderhalden to determine the nature of the proteolytic ferments in the various organs, whether they contain a single enzyme or a mixture. Commercial trypsin, or a dried extract of the pancreatic gland, has already been seen to act differently from pure pancreatic juice, and the same applies to pure gastric juice and commercial pepsin. Glycyl-l-tyrosine is a very convenient substance to distinguish between pepsin and trypsin, as it is not split by the former, but hydrolysed by the latter to glycine and tyrosine. By its means duodenal juice has been shown to contain a ferment like pepsin, since it was not hydrolysed. The ferments of the liver, muscle, kidney contained in the juice obtained by Buchner's method of grinding with sand, mixing with kieselguhr and pressing out at 300 kilograms pressure, behaved like trypsin, but were more active, as they hydrolysed dl-leucylglycine, glycylglycine, glycylalanine, etc. They are therefore less selective in their action, and consequently polypeptides contained in peptone absorbed from the intestine may yet be hydrolysed and assimilated by the various organs. The resultant action of trypsin upon proteins is the production of amino acids and a complex polypeptide (antipeptone of Kühne). This polypeptide, on absorption, will be broken down and be assimilated by the different organs.

The resistant polypeptide may contain other kinds of combination, such as ether, ester (Warburg has found, however, that trypsin acts upon leucinester), diketopiperazine, which are not broken open by trypsin or pancreatic juice, but by the ferments of the liver. The action of arginase upon arginine, a combination of ornithine and urea, which is not acted upon by trypsin, as also the changes which the purine bases undergo in the liver by special enzymes are examples of this kind. In the animal body, the combined action of ferments plays a great part. The resistant polypeptide does not appear in quite the same form when the protein is acted upon first by pepsin and then by trypsin. According to Cohnheim hydrolysis by pepsin followed by erepsin, the ferment obtained from the mucous membrane of the duodenum, produces the same result as pepsin followed by trypsin. This ferment has been shown by Abderhalden to hydrolyse polypeptides not acted upon by trypsin.

It would seem, therefore, as if the study of the action of pancreatic juice would not lead directly to the exact manner of combination in the protein molecule, but it will, nevertheless,

give us a very good insight as to a very large number of these combinations. The only way to arrive at the ultimate solution is the isolation of various complexes before complete hydrolysis. Pick and Zunz have separated various proteoses and peptones, but these are still too complex, as also are the bodies, called kyrines, prepared by Siegfried. We must determine the combinations of two, of three, of four, as they exist, and their possible combinations with each other. A commencement has already been made by Fischer and Abderhalden, who have isolated three dipeptides.

A great deal of confusion has for a long time existed concerning the nature of the proteolytic ferments contained in germinating seeds and various plants. By investigating the action of these enzymes upon various polypeptides, Abderhalden and Schittenhelm found the enzymes of the germinating seeds of wheat and lupines contained a tryptic ferment; and Abderhalden and Teruuchi showed that the endotryptase of yeast and pepsin were like trypsin in their action, whereas the juice of *Nepenthes* contained an enzyme like pepsin in its nature.

V. The Isolation of Polypeptides from Proteins

In 1902 Fischer described the results of some experiments which he had made in conjunction with Bergell upon the isolation of a dipeptide from silk by means of the combined action of acid, alkali, and trypsin. By the action of hydrochloric acid upon silk fibrin, a peptone is obtained which gives a marked reaction for tyrosine. If this be treated with trypsin, the tyrosine is completely split off, and another peptone free from tyrosine results, which when acted upon by baryta, loses ammonia and yields a dipeptide. This dipeptide was considered to be glycylalanine, though the proof could not be satisfactorily given at the time.

This product was not again obtained until quite recently, as the exact conditions of its formation could not be repeated; Fischer and Abderhalden then isolated it in the form of its diketopiperazine. The silk fibrin was acted upon either by sulphuric acid followed by trypsin or by hydrochloric acid; the product was esterified and distilled in the ordinary manner at a low temperature, whereby the esters of the monoamino

acids are removed and the residue treated with ammonia. A substance crystallised out, which was found to be identical with the diketopiperazine formed from glycine and alanine. On hydrolysis it yielded glycine and d-alanine, but whether it was glycyl-d-alanine or d-alanylglycine could not be exactly determined, but most likely it is the anhydride of glycyl-d-alanine, as the product is not hydrolysed by trypsin.

At the same time another dipeptide, glycyl-l-tyrosine, was mentioned, but its constitution was only determined later. Its anhydride has been found to be identical with the synthetical glycyl-l-tyrosine anhydride, and it occurred mixed with the glycyl-d-alanine anhydride obtained in the above manner. Glycine and l-tyrosine resulted on hydrolysis.

By the same method, the anhydride of glycine and leucine has been prepared from elastin; it corresponded exactly to glycyl-l-leucine anhydride. To these three dipeptides, a fourth must be added. It is described by Levene and Beatty as prolylglycine anhydride and was obtained from gelatine.

Further, a crystalline substance, probably a dipeptide, which gives proline and phenylalanine on hydrolysis, has been isolated from the products of the acid hydrolysis of gliadin by Osborne and Clapp.

The protones, which are obtained from the protamines by digestion, have a molecular weight of about 420. Just as in the protamines, eight-ninths of their nitrogen is in the form of arginine. Kossel therefore believes that they are mixtures of tripeptides, such as diarginylalanine, diarginylserine, etc., which have the molecular weights of 401 and 417 respectively.

Leucinimide, leucylleucine anhydride, described many years ago by Bopp, has not yet again been isolated, and, according to Fischer, its existence is doubtful.

The work of Emil Fischer emphasises most strongly the importance of the knowledge of organic chemistry to physiological chemistry, in which branch the proteins are generally studied. There is in reality no absolute difference between the two subjects; physiological chemistry deals with the question how these substances are made, and what changes they undergo, *in nature*, whereas organic chemistry treats of the changes they *can* be made to undergo by various chemical means in the laboratory, and how they can be synthesised. Synthesis also occurs in nature, but whether it proceeds in a manner similar to

that which Fischer has so subtly carried out has still to be determined. The knowledge of the properties of simple definite chemical compounds has enabled us to separate the amino acids, and thus gain an insight into the ultimate structure of a protein. The study of the polypeptides of definite constitution has led to the isolation of such compounds directly from proteins, and will no doubt help us in finding out the nature of the more complex substances, known as proteoses and peptones. The chemical changes which have given us the most knowledge are the hydrolytic changes, and these are produced by the proteoclastic enzymes, which are more delicate and specific in their action than either acids or alkalies. The study of the action of enzymes upon the proteins and the polypeptides gives us indications as to how the protein may eventually be synthesised, by directing the manner in which we combine together the amino acids to form simple polypeptides, these again into greater complexes, and so on until a protein results. We have seen from the above that this method is being carried out, polypeptides having been not only synthesised, but also isolated directly from the protein, and their identity established.

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SOME RECENT RESEARCH UPON THE BIRTH AND AFFINITIES OF CRYSTALS[1]

By PROFESSOR H. A. MIERS, D.Sc., F.R.S.

The main facts concerning the growth of crystals are familiar to all, and do not require more than the briefest statement. A saturated liquid solution of any substance, A, in any other, B, is a mixture of A and B in the liquid condition, such that if a solid crystal of A be immersed in it the crystals will neither grow nor dissolve. If the solution be ever so slightly weakened by addition of B, the crystal will begin to dissolve till equilibrium is restored; the same effect is in general produced by slightly warming the solution. If, on the contrary, the liquid be ever so slightly supersaturated by removal of B (*e.g.* by evaporation), the crystal will begin to grow till equilibrium is restored; the same effect is in general produced by slightly cooling the solution.

The solubility or freezing-point curve, SS (fig. 1), gives the temperature at which a series of the solutions of different strengths are saturated. It may be experimentally determined by immersing a minute crystal fragment of A in each solution as it cools, and noting the temperature at which the fragment neither grows nor dissolves. If the solution be cooled below the saturation temperature without the insertion of A, and if it be protected from agitation and from exposure to the air, it may often be preserved for a long time in the supersaturated condition without crystallising. Its behaviour is in this respect similar to that of a simple liquid supercooled below its freezing point; the latter can also be preserved for a long time in the supercooled condition without crystallising. The insertion of a fragment of the solid crystalline substance in either at once

[1] This paper embodies the substance of two lectures delivered at the Royal Institution on April 11 and 18, 1907.

causes crystallisation to begin, and the same effect may often be produced by shaking.

> Break thou deep vase of chilling tears
> That grief hath shaken into frost,

are well-known lines from *In Memoriam* that refer to this remarkable effect.

It is curious that no systematic observations have hitherto been made upon the behaviour of solutions which are continuously shaken while cooling.

In the course of some experiments upon the refractive indices of cooling supersaturated solutions of various salts made by Miss Florence Isaac and myself in the Mineralogical Laboratory at Oxford we noticed that when such a solution is stirred in an open vessel, a thin shower of crystals generally appears at or about the saturation temperature; but that after the temperature has fallen about 10° below this point there ensues a dense shower of crystals, which is generally so thick as to make the liquid almost opaque. The sudden weakening of the solution at the lower temperature is accompanied by a sudden fall of the refractive index, which attains its maximum value at this temperature.

On the first day, when we experimented with a solution which we had not previously used, the earlier and thinner showers of crystals did not appear, and the later and denser shower might be somewhat retarded. But in subsequent experiments the thin shower (accompanied by a slight diminution in the rate of increase of the refractive index) occurred at exactly the saturation temperature, and the dense shower (accompanied by an actual diminution of the refractive index) occurred at a perfectly definite lower temperature.

We infer that the first shower takes place because some fine crystalline particles of the dissolved substance get into the solution with the dust of the air, and continue to grow, and that the second shower is due to a different cause.

If this be so, then the same solution enclosed in a sealed glass tube should not yield the first shower.

We have made this experiment with numbers of aqueous solutions of different substances and of different strengths, and have always found that in a sealed tube the first shower is prevented, and that the liquid cannot be made to crystallise

at a higher temperature than that corresponding to the appearance of the second shower in an open vessel. For example, a 48·2 per cent. solution of sodium nitrate is saturated at 26°, and yields the dense shower at 16°; a 51 per cent. solution is saturated at 39°, and yields the dense shower at 30°; and these solutions enclosed in sealed tubes only yield a shower on shaking at 16° and 30° respectively.

During the visit of the British Association to South Africa

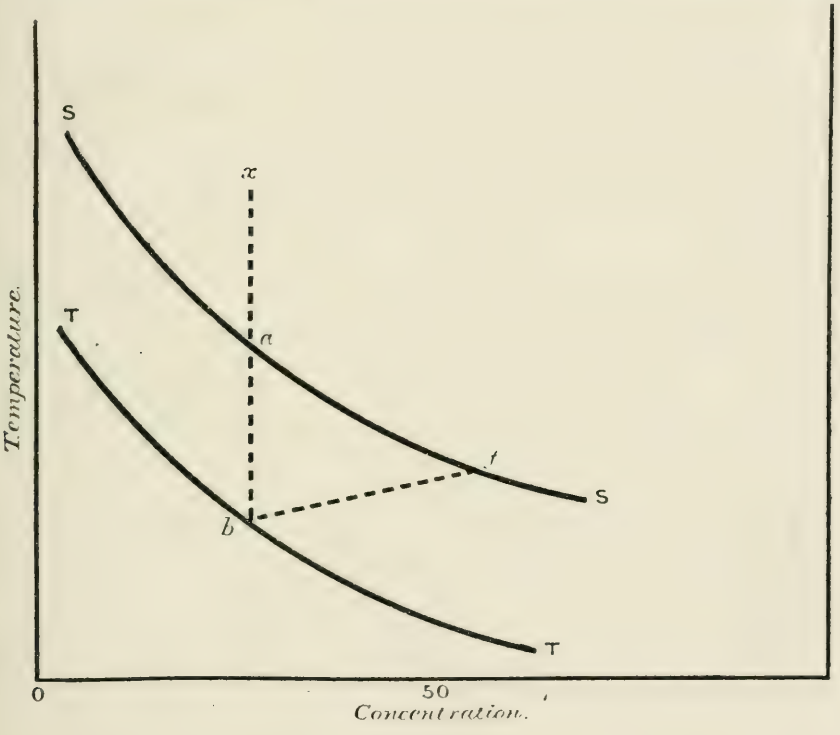

FIG. 1.

I carried sealed tubes containing these and other solutions with me, and during several weeks kept the first solution between the temperatures of 26° and 16°, and shook it violently at intervals without causing crystals to appear. But as soon as the temperature fell to 16° the solution began to crystallise.

We conclude, therefore, that in an open vessel such a solution crystallises at 26° only because the liquid is inoculated by crystal germs from the air, and that it crystallises again at 16° because fresh crystals appear spontaneously or are born in the

liquid. At 26° the cooling liquid passes from the unsaturated condition to a supersaturated one, in which crystals can grow, but require to be introduced from without; at 16° it passes into a condition in which crystals can be made to appear in the liquid by mechanical means such as shaking. Between these temperatures the solution is in what Ostwald has termed the "metastable" condition; below 16° it is in what he terms the "labile" condition: and accordingly we distinguish the two showers, the first by the name "metastable shower" and the second by the name "labile shower."

In any laboratory the air must contain innumerable particles of every material that is being employed in the laboratory, and, so far as we know, immeasurably small germs are sufficient to inoculate a supersaturated solution and make it crystallise; in fact, the crystallisation of an exposed solution must be an extraordinarily sensitive test for the presence of such crystalline germs.

If now the exact temperature at which the dense labile shower makes its appearance be determined for a series of solutions of different strengths, the observations will trace on the temperature-concentration diagram a new line, which we call the "supersolubility curve," giving the relation between temperature of spontaneous crystallisation and strength of solution; this is the curve TT in fig. 1.

We have determined this curve for various solutions such as sodium nitrate and sodium chlorate. We have also, by observations upon the refractive index, traced the changes of concentration in a cooling solution, and we find that it is not possible to obtain a clear representation of what actually occurs without taking account of the supersolubility curve. It is customary to think of a cooling solution as beginning to crystallise so soon as it becomes supersaturated, and as crystallising at such a rate that it remains just supersaturated and no more. If this were so, the changes in the temperature and constitution of the liquid would be represented by the line *xaf*. In reality, unless the cooling be very slow, the order of events is more faithfully represented by the line *xabf*; a few crystals appearing by inoculation at *a* and continuing to grow very slowly, whereas a very rapid growth, which is generally accompanied by a cloud of crystals if the liquid be shaken, sets in at *b*. Messrs. Hartley and Garrod Thomas have traced

the supersolubility curves for triphenylmethane in organic liquids, and have shown how they explain the course of crystallisation even when that substance forms crystalline compounds with the solvent. These authors suggest that the metastable condition is to be explained by the increased solubility which is known to belong to very small crystals. The first products of spontaneous crystallisation must accordingly be more soluble than the larger crystals for which the solubility curve is determined. They cannot therefore persist through the early stages of growth until the degree of supersaturation becomes sufficient to counteract the solubility.

Many peculiarities or apparent anomalies in the crystallisation of solutions can be explained when account is taken of their passage from the metastable to the labile condition.

An experiment familiar to every schoolboy is the scratching of the inside of a test tube containing a strong solution with a glass rod, and the appearance of the first crystals along the line of scratch. This is often explained as due to a tendency of crystals to grow on rough surfaces and protuberances rather than on a flat, smooth surface.

If this explanation were correct, it ought to make no difference whether the scratch be made before or after the tube is filled with the solution, and the crystals ought to appear along the lines of old scratches. In reality, it is necessary that the scratch should be made within the liquid; moreover, we have found that no effect is produced until the solution has been supersaturated to the exact point at which it is becoming labile. The process of scratching merely supplies the mechanical disturbance sufficient to make the crystals grow in the labile solution.

It is true that scratching may produce a line of crystals in a metastable solution; but this only occurs if the rod is introduced from outside, and has caught up crystalline germs in the air or at the surface of the liquid; no effect is produced by scratching with the end of a rod which has been kept in the metastable liquid throughout the cooling.

Now a strong aqueous solution, being merely a mixture of the dissolved substance and of ice, both in the liquid condition, a similar supersolubility curve ought to be obtained for the spontaneous freezing of ice in solutions which contain excess of water. We have not conducted any experiments at sufficiently

low temperatures to trace this curve. But we may confidently predict that for an aqueous solution of a salt which separates in the anhydrous condition—or for a mixture of any two substances which do not form compounds or isomorphous mixtures—the usual V-shaped freezing-point curve of the two components (ME*m* of fig. 2) is accompanied by a V-shaped supersolubility curve (SH*s* of fig. 2) representing their temperatures of spontaneous crystallisation.

[Since writing the above I hear from Mr. Hartley that he and Mr. Barrett have realised the ice supersolubility curve in studying the crystallisation of dilute solutions of sodium sulphite.]

In general, therefore, the cooling of two substances dissolved in each other, whether an ordinary solution, a mixture of two fusible substances, a rock, or an alloy, will be represented by fig. 2, in which M and *m* are the melting points of the two substances A and B, ME and *m*E their freezing-point curves meeting in the eutectic E; S and σ are the temperature of spontaneous crystallisation of A and B when molten and supercooled; SH and σH constitute their supersolubility curve, meeting in what we call the "hypertectic" point H; this is the highest temperature at which they can crystallise spontaneously together.

The process of cooling of a liquid mixture initially of composition X is generally supposed to be represented by the line *xaf*E. The liquid cools down till it becomes saturated at *a* and then cools, with crystallisation of A, always remaining saturated (as represented by *af*E) until at E it reaches the eutectic composition, when B and A can crystallise together without change of temperature, and the whole mass solidifies as the eutectic surrounding the crystals of A.

It will now be clear that this only occurs if the liquid be inoculated with A, be kept stirred, and be cooled extremely slowly. Otherwise supersaturation will occur, and the process is, again, more faithfully represented by the line X*abf*E; crystals may make their appearance by inoculation at the temperature *a*—but at the temperature *b* there will be a sudden access of crystallisation by reason of the spontaneous growth, and some time will elapse before the solution again falls to saturation point at *f*. Or, before this happens, the solution may become labile for B, and that substance may crystallise spontaneously, and so the eutectic mixture may never be attained.

In order to test these predictions we have made experiments

with mixtures of two salicylates, salol and betol, choosing these substances merely because they melt at convenient temperatures, and we have succeeded in tracing for them the complete curves like those shown in fig. 2. By means of the refractive index we have traced the changes in each solution as it cools, and have always obtained curves resembling *abf* of fig. 1. We

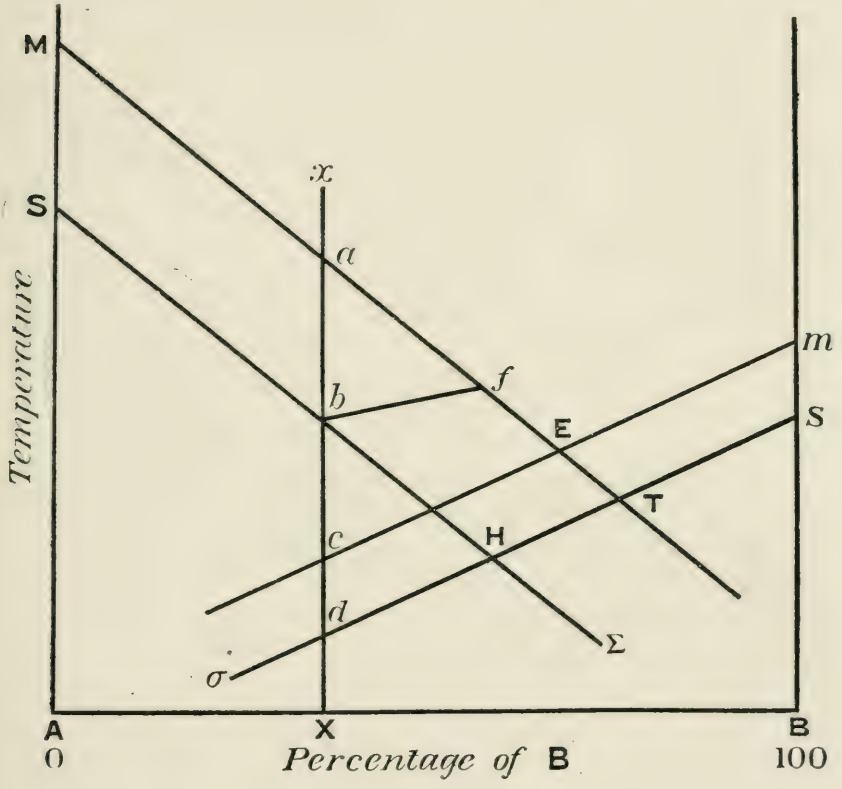

Fig. 2.

have also found that, as is clear from the figure, any mixture has no less than four possible freezing points—namely, two temperatures, *a* and *c*, at which A and B crystallise respectively by inoculation, and two others, *b* and *d*, at which A and B crystallise spontaneously.

The same general principles must hold for mixtures of more than two components; we have already traced the super-solubility curves for mixed solutions of sodium nitrate and lead

nitrate, *i.e.* for mixtures of three components, the two salts and water.

No doubt the same principles prevail in the solidification of metals, alloys, and rocks, although the problem is here complicated by the formation of compounds and isomorphous mixtures (solid solutions); but whatever substance solidifies, it can only crystallise from a solution which is supersaturated with regard to it, and unless this is made to take place by inoculation, the solution must first become sufficiently supersaturated to crystallise spontaneously.

The freezing-point curves terminate at M and *m* in the melting points of the pure substances A and B; and the supersolubility curves terminate at S and *s*, the temperatures of spontaneous crystallisation of A and B. We have determined these for salol and betol. We have also made experiments with water, and have found that pure water contained in sealed tubes crystallises by shaking at $-1{\cdot}9^{\circ}$ C.; and even if heavy substances be enclosed with the water in the tubes so as to produce friction, we have been unable to make it crystallise at a higher temperature than $-0{\cdot}4^{\circ}$ C.

If the crystallisation of a drop of any solution be watched under the microscope, remarkable changes in the process will generally be noticed so soon as the drop passes from the metastable to the labile condition. Experiments made in my laboratory by M. Chevalier have shown that, in a drop of labile solution, potash-alum crystallises as a rectangular network of fine needles, and not in the ordinary octahedra. This observation accords with the general experience that when crystals grow very rapidly they usually grow as needles. I believe this to be due to the fact that when a crystal has started in a labile solution the liquid in immediate contact with it is at once reduced to the metastable condition both by loss of solute and by rise of temperature, so that the growth only takes place within the sheath of less concentrated liquid with which the crystal envelopes and protects itself, and therefore proceeds quite slowly. But if any point of the crystal has begun to advance so rapidly that it projects through this sheath before the zone of weaker solution has time to close round it, then it may continue to advance rapidly through the strongly supersaturated labile solution; and the concentration currents which play most energetically upon the end of a crystal will ensure

that the point is continually in contact with the strong solution, and therefore that the crystal continues to grow rapidly in this direction and slowly in others. That the liquid in contact with a slowly growing crystal is supersaturated but only metastable, I have proved by actually measuring its refractive index. Again, when a rapidly growing needle approaches a crystal at a part of its surface which is growing slowly, it ceases to grow rapidly just before actual contact takes place—*i.e.* so soon as it enters the zone of metastable solution surrounding the latter.

If a thin drop of solution of potassium bichromate be watched as it crystallises on a warm slide, the ring of crystals which form on the edge of the drop will be seen to advance rapidly in a beautiful growth of needles; the process is then suddenly arrested, and the ring continues to grow quite slowly with a thickening of the crystals. After a short interval the sudden growth is repeated, and is then as suddenly checked; and so on until the drop is dry. The explanation is probably the following: the labile solution in which the crystals first appear is reduced in strength by their rapid growth, and falls to the metastable condition, in which the growth is slow—evaporation then raises the strength to the labile point; rapid growth again sets in, and the process is repeated.

Other illustrations of the same recurrent process are to be found (1) in "Liesegang's Rings" of silver chromate produced by a solution of silver nitrate creeping through gelatine which has been impregnated with potassium bichromate; (2) in the stripes of silver chloride deposited in nerve fibres by the operation of acid nitrate of silver acting upon the chlorides in the fibre, as described by Macallum, and in capillary tubes as described by Boehm.

Another feature of crystals growing rapidly in a labile solution is that they are not only apt to grow in long prisms and needles, but to group themselves into the branching and tree-like forms which lend such a beautiful appearance to many of these growths. For example, the frost pattern on a window pane, and the rapid growths of potassium bichromate just described.

This branching of needles is generally due to "twinning," the process by which two crystals grow out of each other in different directions at a fixed angle which depends upon the angles of

their own facets. Twinning can generally be explained as a union of the two crystals along a plane face which is the same in the two crystals, but is reversed in one of them, so that the two crystals grow, as it were, back to back. (Sometimes it is an edge which is reversed.) It is highly probable that twinning is due to the fact that the structures which build up the two crystals can fit together in two positions, either parallel, or the one reversed with regard to the other.

In the rapid and turbulent growth in a labile solution it may well happen that this reversal is more apt to take place than in the more placid growth in a metastable solution. Hence the beautiful stellate groups and tree-like forms which are more characteristic of rapid than of slow growth.

Now just as two crystals of any substance may grow on each other whether in parallel position or in twin (reversed) position, because they have the same structure and fit together in these positions, so it may happen that two crystals of different substances may grow together in parallel position for the same reason. This is well known in such groups as that of the alums, where one alum, if introduced into the solution of another alum, may continue to grow as a single crystal, and become enveloped by the latter—as is clearly visible when the two are of different colours. In such a case the two substances are absolutely similar in chemical composition, only differing by the substitution of metals which exhibit the greatest resemblances in chemical behaviour; and this is no doubt the reason why they possess identical structures. Another still more remarkable case is that of calcite and sodium nitrate. A drop of strong solution of sodium nitrate placed on a clean cleavage surface of calcite at once deposits small rhombohedra which are parallel to one another, and to the rhombohedron on which they are growing. The angles of sodium nitrate are very near to those of calcite, but not identical with them. Further, there is clearly a certain similarity of composition in $CaCO_3$ and $NaNO_3$.

In the course of some systematic experiments upon the parallel growths of different substances, carried out in the Mineralogical Laboratory at Oxford, Mr. T. V. Barker investigated the question whether sodium nitrate also grows in parallel position upon the other minerals of the calcite group, the carbonates of iron, zinc, magnesium, and manganese. He found that

calcite is the only one which possesses this power of bringing down sodium nitrate in parallel position upon itself. Yet calcite is by no means the one which most nearly resembles sodium nitrate in form; in fact, it differs from it in angle more than any of the other carbonates. It is clear then that some other criterion than equality of angle must be applied. On comparing the molecular volumes, however, it is found that calcite is the only one mineral in this group which possesses a molecular volume nearly equal to that of sodium nitrate. A reason why this equality of molecular volume should be essential for a parallel growth to take place is made evident from the following considerations.

In order that two structures of particles may fit together it

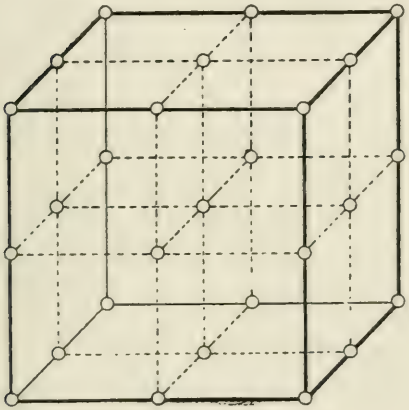

Fig. 3.

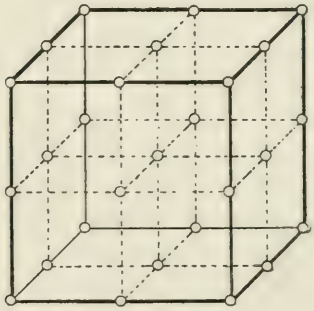

Fig. 4.

is necessary not only that the arrangement of the particles should be the same in both, but also that the spacing of the particles should be identical. Consider, for example, a cubic structure consisting of particles arranged as in fig. 3. The particles are situated at the corners of a series of equal cubic cells. Let fig. 4 be a similar structure, but smaller in dimensions. Both 3 and 4 are cubic structures, and will yield crystals having the same angles; but they will not fit together, because the cubic cells of 3 are larger than those of 4. If the structures are to be identical they must be made up of cells whose volume is the same.

Now the specific gravities, or relative weights of equal volumes, of the two crystals will depend both upon the intrinsic

weight of the particles at the corners of a cubic cell and upon the volume of the cell, and will vary directly as the former and inversely as the latter. The former is, of course, given by the molecular weight—the weight of the atoms of which the molecule consists. If, therefore, the two crystals are to have cells of the same volume, the molecular weight divided by the specific gravity (*i.e.* the molecular volume) must be the same for both. The same principle applies to any two similar structures—not necessarily cubic. The equality of molecular volume accounts, therefore, for the fact that sodium nitrate crystallises in parallel position on calcite alone. That they do possess similar structures is indicated by their similarity of angle, cleavage, and optical and other physical properties.

If this be a true explanation, it must hold good for other groups. Now Mr. Barker has discovered that the alkaline perchlorates and permanganates have the same structure as the mineral sulphates of lead, strontium, and barium (anglesite, celestine, and barytes), as shown by their similarity of angle, cleavage, and constitution.

Among the nine salts examined he found only two, namely, $KClO_4$ and $KMnO_4$, which readily yielded parallel growths on barytes $BaSO_4$ and the other two minerals of the barytes group, and these are precisely the salts which possess nearly the same molecular volumes as the sulphates.

A yet more extensive series has been studied by Mr. Barker, the alkaline haloids which crystallise in the cubic system. No less than seventeen of these were examined, and among the two hundred and seventy-two possible combinations realised, parallel growths were only obtained between those which possess most nearly the same molecular volumes.

[Equality of molecular volume in a series of substances having the same crystalline structure may also be expressed as equality of topic axes, a conception which is rapidly becoming familiar since its introduction by Becke, Muthmann, Tutton, and others, and one which enables us to compare not only volumes, but corresponding magnitudes along parallel directions in such a series.]

In all these groups it seems that when the molecular volumes are almost exactly equal, the parallel growth is so perfect and uniform that it takes place over the whole surface of the crystal, which then appears simply to increase in size by the addition

of the new material. This happens with the alums, as mentioned above.

The researches which I have described in this paper give us, I think, some insight into the invisible structure of the crystal edifice and its manner of growth; they also lead to one other inquiry which has not yet been carried out. Parallel growths take place upon crystals of the same substance; they also take place upon crystals of another substance having the same structure and molecular volume. A crystal of the dissolved substance dropped into a metastable solution at once relieves the supersaturation and continues to grow. Is it the case that the supersaturation can also be relieved by a crystal of some other substance, provided that it has the same structure and molecular volume? I think that this will probably be established by future experiments, for I have found that the supersaturation of a metastable solution of sodium nitrate appears to be relieved by calcite, but not by the other members of the calcite group.

If this be so, the relief of supersaturation and the parallel growth are two occurrences which can hardly be referred merely to the geometrical fitting together of two structures, for this could scarcely force the dissolved substance out of solution. I would rather think of a crystal as an assemblage of vibrating particles, and of the geometrical structure as the expression of some rhythmical motion with which they are endued, so that two crystals which have similar structures are in tune with each other, and respond each to the other's palpitations. Is it not possible that the irregular motions of the liquid substance in solution may respond to the rhythmical motion of the crystal particles of a kindred substance in tune with it introduced into the solution, just as one violin responds to another, and so may be transformed into the regular pulsations which perhaps accompany the building up of a crystalline structure?

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DOUBLE SALTS

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Amongst the substances possessed of the typical properties summarised in the name "salt," chemists have for long known and dealt with a large number of comparatively greater complexity of composition, due to their having been produced from two "simple salts." These "compound salts" are generally well crystallised, and their formation from the simple salts is usually accompanied by elimination or absorption of water of crystallisation (Table II.). As a rule, the two constituent simple salts have a common ion: either the anion, as in the case of two sulphates (*e.g.* K_2SO_4 and $Al_2(SO_4)_3$, $FeSO_4.7H_2O$ and $MgSO_4.7H_2O$), or of two cyanides (*e.g.* KCy and AgCy, KCy and FeCy); or the cation, as in the case of two salts of sodium, or of rubidium, or of potassium (*e.g.* $NaNO_3$ and Na_2SO_4; $Rb_2d-C_4H_4O_6$ and $Rb_2l-C_4H_4O_6$; $HClO_4$ and $KMnO_4$). But instances are known in which all the four ions are different (*e.g.* $Cd_2K_4(C_2O_4)_3Cl_2$). The formulæ

$$mMA + nM'A$$
$$mMA + nMA'$$
$$mMA + nM'A'$$

represent these different types of combination. Of these only the two first, *i.e. compound salts formed from simple salts which have a common ion*, fall within the scope of the subject of this paper, and it may here be noted incidentally that in the predominating number of cases the common ion is the cation. Such combinations divide themselves into three classes, sharply separated from one another (Table I.).

I. The ratio of the quantities of the two constituent simple salts varies continuously, with or without a break in the series, from MA = 0 and M'A = 100 per cent. to MA = 100 and M'A = 0 per cent.

In order to form such compound salts the constituent simple

salts must be isomorphous. The series of combinations thus formed, named *mixed crystals*, are of the nature of *solid solutions*; their physical properties are continuous functions of the composition; their chemical properties are those of the two constituent simple salts, *i.e.* the analytical reactions are those of the ions of the two simple salts. Thus $KClO_4$ and $KMnO_4$ are isomorphous and form mixed crystals of all shades of depth of colour, from almost colourless through pink to deep purple, according to the relative amounts of colourless $KClO^4$

TABLE I

CLASSIFICATION OF COMBINATIONS mMA . nM′A FORMED FROM TWO SIMPLE SALTS MA AND M′A

I. MIXED CRYSTALS	II. DOUBLE SALTS	III. SALTS OF COMPLEX ACIDS
(1) *Composition*		
Series from $m = 100, n = 0$ to $m = 0, n = 100$	m and n = simple whole numbers.	
(2) *The* **physical** *properties of the* **solid** mMA . $nM'A$ *are:*		
additively those of the constituent salts, continuous functions of the chemical composition in terms of the simple salts.	in no definite way related to those of the constituent simple salts.	
(3) *The* **chemical** *properties of* **solutions** *of* mMA . $nM'A$ *are:*		
those of the ions of the simple salts, *i.e.* those of M, M′, and A.		*not* those of the ions of the simple salts MA and M′A, but of ions M and $M'A_{m+n}$.

and deep purple $KMnO_4$ present in the crystals. The same additive relation holds for other physical properties, such as specific volume, refractive index, etc. Moreover, a solution of the mixed crystals will give the reaction for the potassium cation and the perchlorate and permanganate anion. Retgers,[1] through his investigations of the relation between the physical properties and the composition of whole series of such salts, has supplied us with definite principles whereby to recognise *mixed crystals*, the chief interest attaching to this class of complex salts being in connection with the study of isomorphism.

[1] "Zur Kenntnis des Isomorphismus," I.–XII., *Zs. physik. Chem.* 4–20, 1889–1896.

II. The ratio of the quantities of the two component salts is a simple molecular one; in mMA.nM′A m and n are very simple whole numbers. Obviously any series of *mixed crystals* must comprise members the composition of which also exhibits such a simple molecular ratio, and cases of confusion have in consequence not been infrequent; but there is the distinguishing feature that whilst for mixed crystals a variation in the proportion of the simple salts present in the solution from which they are deposited will produce a series of gradually varying composition, in the class now under consideration the number of combinations obtained is very small, generally only one; and when it is more than one, the change in composition is sudden, m and n remaining simple whole numbers. Moreover, in this second class the physical properties, such as colour, specific volume, crystalline form, solubility, etc., etc., are not in any definite way related to those of the constituent simple salts, but generally differ widely from these Thus, to take as an example the easily apprehended property of colour, the following constitute striking cases: white acetate of calcium and green acetate of copper, if brought together in equi-molecular quantities, yield a magnificently deep blue salt of the formula $CaCuAc_4.8H_2O$; colourless LiCl and green $CuCl_2.2H_2O$ give a compound of the formula $CuLiCl_3.2H_2O$, which, in a spirit of unusual rebellion against the predominant blue and green of cupric salts, crystallises in garnet-red needles; and similar examples might be multiplied. Compound salts of this type, when in solution, give the analytical tests of the ions of the constituent salts, and they are termed *double salts*, a name which until comparatively lately was indiscriminately applied also to that kind of complex substances formed from two simple salts which constitutes the third and last class.

III. In this class, as in the preceding one, the ratio of the quantities of the two component salts is a simple molecular one; but the compound salt is distinct from a mixture of the components not merely in respect of the physical properties of the *solid*, but also in respect of the chemical properties of the *solution*. Typical examples of such cases are

$$4KCy + FeCyl_2 = K_4FeCy_6$$
$$2KCl + PtCl_4 = K_2PtCl_6$$
$$3K_2C_2O_4 + Cr_2(C_2O_4)_3 = 2K_3Cr(C_2O_4)_3,$$

and it is not necessary to repeat here the evidence for the assertion that in all these compound salts the analytical reactions are not those of the simple salts from which they are formed, or—in modern terminology—that in solution these compound salts have ions different from those of the simple salts from which they are formed. Ostwald, to whom we owe the clear recognition of the characteristics of this type of compound salts and their separation from double salts proper, says: "The name of double salts may not be given to any combination of two salts which gives reactions different from those of the constituents."[1] This was written in 1889. The separation insisted upon is definite and legitimate, and the consequent definition of *double salt* and *salt of complex acid* respectively is easy; yet the principle has been slow in getting fully recognised in standard text-books. In the last (that is, the 1897) edition of the volume of Roscoe and Schorlemmer dealing with metals, we find a definition of double salt which it would be difficult to beat for vagueness.

"The term double salt is applied somewhat loosely to salts formed by the combination of one molecule of one salt with one or more molecules of another salt or of an acid, etc. Thus the substances represented by the formulæ $KF + HF$, $AL_2(SO_4)_3 + K_2SO_4 + 24H_2O$, etc., are called double salts. Very little is known about the constitution of such substances, since they are generally decomposed, at all events to some extent, when they are dissolved in water, although they separate out again unchanged when the solution is concentrated. In many cases they are undoubtedly salts of complex acids or bases."

Does not the suspicion obtrude itself that the vagueness is intentional? Vagueness of any kind cannot be brought as a charge against another writer, who does not follow the rule laid down by Ostwald. Mendeleeff, in a piece of vigorous writing, absolutely repudiates the conception of complex acid radicles, considering it unnecessary. The following is a quotation from the 1905 (the third) English edition of his *Principles of Chemistry*, taken from the paragraph dealing with K_4FeCy_6 and K_3FeCy_6.

"Of these two remarkable and very stable salts, it must be observed that with ordinary reagents neither of them gives the same double decompositions as the other ferrous and ferric salts, nor exhibits the characteristic properties of the potassium

[1] "Dissociationstheorie der Elektrolyte," *Zs. physik. Chem.* 3, 1889, p. 598.

cyanide which they contain." Then, after enumeration of the well-known differences, he continues: "This led the earlier investigators to recognise special independent groupings in them. . . . Such a representation is, however, completely superfluous for the explanation of the peculiarities in the reactions of such compounds as double salts. . . . Every double salt ought to be regarded as a peculiar kind of saline compound; potassium cyanide is, as it were, a basic, and ferrous cyanide an acid element. They may be unstable in the separate state, but form a stable double compound when combined together; the act of combination disengages the energy of the elements, and they, so to speak, saturate each other."

Probably most readers would fail to discover in the above a precise meaning or any meaning at all, and the eminent author must himself have had some misgivings on the subject, as he proceeds to say:

"Naturally, all this is not a definite explanation; but then the admission of a special complex radicle can even less be regarded as such."

With the second part of this statement it is not possible to agree, and I have quoted this view concerning the denotation to be given to the term "double salt" only on account of the prestige of its author, and not because it is one which, as far as I know, has found other supporters. We may therefore consider the term "double salt" to be applied only to such crystalline solids, obtained by the combination in a simple molecular ratio of two salts with generally a common ion, as have physical properties different from those of a mixture of the constituents of the same composition, whilst the chemical properties of a solution of the double salt differ in no way from the sum of those of the constituent salts.

In the memoir of Ostwald already referred to, in which "double salts" were for the first time clearly differentiated from the "salts of complex acids," it was further stated as a characteristic of double salts that their existence is limited to the solid state, and that in solution not only their chemical but also their physical properties are additively those of the constituent salts. This, though true as a limiting case for double salts of slight solubility which are practically completely dissociated in solution, has not been found to hold generally; and it has been possible to supply extensive and varied experi-

mental proof for the existence in solution of double salts, an existence which moreover one would be led to expect theoretically. The methods used in these proofs of course all depend on the comparative measurement of physical properties, the values found for the solution of the double salt XY being compared with those calculated for a mixture of the constituent simple salts from the experimental values obtained for X and Y separately.

The following are examples of such comparative measurements :

(i) *Electrical Conductivity.*—Measurements have been carried out by many investigators, who found that in moderately strong solutions, N and N/2, the double salts are but little dissociated. Thus the differences between the conductivities of $2KCl . ZnCl_2$ and of $2KCl + ZnCl_2$ (calculated) amount to nearly 36 per cent., which greatly exceeds the value of the difference which would be due to the influence on one another of salts containing a common ion.[1]

(ii) *Lowering of the Freezing-point.*[1]—For $2KCy + HgCy_2$ the calculated molecular lowering is by 4·77° greater than that observed for $K_2Hg(Cy)_4$, corresponding to a disappearance of about two and a half molecules (or ions) in the formation of one molecule of the double salt.

Calculated from $2KCy + HgCy_2$.	7·57 + 1·93 = 9·50
Found for K_2HgCy_4	= 4·77
Difference . .	4·73

(iii) *Rotation of the Plane of Polarisation.* — Measurements have shown that, whilst for solutions containing mixtures of salts known not to yield double salts the effect measured is additive, solutions containing substances such as 2KI and HgI_2 show marked deviation from the additive values.[2]

(iv) *Colour.*—The yellow chloride of iron and the colourless chloride of potassium form at 22° C. and above a double salt of the composition $FeCl_3 . 2KCl . H_2O$, which in the solid state is red. A concentrated solution of the mixture of the two simple

[1] Jones, H. C. (and others), "Contributions to our knowledge of the Aqueous Solutions of Double Salts," *Amer. Chem. J.* 22, 1899, p. 5 ; 25, 1901, p. 349.

[2] Schönrock, "Elektromagnetische Drehung der Polarisationsebene in Flüssigkeiten und Salzlösungen," *Zs. physik. Chem.* 11, 1893, p. 776.

chlorides is yellow at ordinary temperature, showing the characteristic colour of $FeCl_3$; on raising the temperature to about 25°, the colour changes to red, indicating the presence in the solution of the double salt.[1]

These various investigations, taken in conjunction, justify the inference that the existence of double salts in solutions of definite concentration has been established.

All said so far has been solely classificatory and has only brought us to the recognition of the distinctive properties of double salts. Quite separate from this is that side of the study of double salts which since 1887 has stimulated so much interest and has produced so great an output of work. Any historical review of the recent growth of physical chemistry, of the extension of its scope and the deepening of its foundations would doubtless have to say something about the 1887 volume of the *Zeitschrift für physikalische Chemie*; and that not only, or not even chiefly, because this was the first volume of the journal which, initially a sign of the rapidly increasing activity in a special branch of chemistry, has become so instrumental in stimulating that activity, but because in it are contained papers which have already become classical as the starting-point and the foundation for new and most important theories.

In that volume Van't Hoff wrote on "The Part Played by Osmotic Pressure in the Analogy between Solutions and Gases," Arrhenius on "The Dissociation of Substances Dissolved in Water"; and in it appeared another contribution by Van't Hoff entitled "The Transformation Temperature in Chemical Decomposition." This last-named paper was the first in a long series of experimental and theoretical investigations, the results of which are summarised in the book published by Van't Hoff in 1897 under the title *The Formation and the Splitting of Double Salts*. Starting from the observation that the formation of a double salt from its components and the reverse change occur at a definite temperature, above which temperature only the double salt and below which only the two constituent simple salts can exist, or *vice versa*, Van't Hoff, in the pioneer paper referred to, gives those examples which now are found in every text-book of physical chemistry (Table II.). When the simple crystalline

[1] Hinrichsen and Sachsel, "Über die Bildungs- und Löslichkeitsverhältnisse der Doppelchloride des Eisens und der Alkalimetalle," *Zs. physik. Chem.* 50, 1905, p. 90.

sulphates of sodium and magnesium are rubbed together and heated to above 22° the mass liquefies as the result of the formation, accompanied by splitting off of water, of the double sulphate *astrakanite*. Conversely, finely powdered astrakanite, when rubbed with a small amount of water at a temperature below 22°, soon solidifies to a hard mass, which is a mixture of

TABLE II

EXAMPLES OF DOUBLE SALT FORMATION AND DECOMPOSITION

	Transition Point and Transition Interval.	Heat effect in formation of D.S.	Change of hydration in formation of D.S.
I. Astrakanite			
$+\begin{matrix}Na_2SO_4 . 10H_2O\\MgSO_4 . 7H_2O\end{matrix}\rightleftarrows Na_2Mg(SO_4)_2 . 4H_2O + 13H_2O$	D.S. *formed* at 21·5° and partially decomposed by water between 21·5° and 25°.	Heat absorbed.	Water split off.
II. Cupric Dipotassium Chloride			
$\begin{matrix}CuKCl_3\\+KCl\\+2H_2O\end{matrix}\rightleftarrows K_2CuCl_4 . 2H_2O$	D.S. *decomposed* at 92°.	Heat evolved.	Water absorbed.
III. Copper Calcium Acetate			
$\begin{matrix}CuAc_2 . H_2O\\+CaAc_2 . H_2O\\+6H_2O\end{matrix}\rightleftarrows CuCaAc_4 . 8H_2O$	D.S. *decomposed* at 75°.	Heat evolved.	Water absorbed.
IV. Seignette (or Rochelle) Salt			
$\begin{matrix}2Na_2Ta . 2H_2O\\+2K_2Ta . \frac{1}{2}H_2O\\+11H_2O\end{matrix}\rightleftarrows 4NaKTa . 4H_2O$	Double tartrate *decomposed* at 55° and partially decomposed by water between 41° and 55°.	Heat evolved.	Water absorbed.
V. Scacchi's Salt			
$d.NaNH_4 . Ta . 4H_2O + l.NaNH_4 . Ta . 4H_2O \rightleftarrows (Na . NH_4 . Ta)_2 . 2H_2O + 6H_2O$	Racemate (double) *formed* at 27° from two tartrates (double), not decomposed by water.	Heat absorbed.	Water split off.

the two constituent sulphates, a change which does not occur if the salt and the water are kept above 22°. He also quotes the case of the green acetate of copper and the white acetate of calcium, which, when powdered finely and moistened with water in the ratio of the equation $CuAc_2 . H_2O + CaAc_2 . H_2O + 6H_2O$, solidify at ordinary temperature to a hard blue mass of the double salt; the latter, when heated to above 78°, again decomposes into the two constituent salts, a transformation which makes itself evident

by the change of colour from blue to green. Van't Hoff recognises these and similar transformations to be of essentially the same kind as those occurring in fusion, of which the reversible change ice-water is the simplest example; or as those occurring in allotropic transformation, of which the reversible change of rhombic to monoclinic sulphur at 96° is an instance. What all these cases have in common is, that the systems concerned are in their composition heterogeneous, *i.e.* made up of non-miscible portions which, themselves homogeneous, are marked off from one another by bounding surfaces, and which can be mechanically separated from one another, such portions having by Willard Gibbs been named *phases*. Moreover, in the changes occurring there is the complete transformation at a definite temperature, named the *transition point*, of one phase into another, and the equilibrium condition is independent of the total amount present of each phase. Thus the formation and the splitting up of double salts—that is, the reversible change of one solid phase into two other solid phases—belongs to the class of *complete heterogeneous equilibria*; and the investigation of the effect on the equilibrium condition of changes of temperature, pressure, and concentration are problems which it has become possible to solve by the application of the phase rule. In the formation of a double crystalline salt which separates from an aqueous solution of the two component simple salts, we deal with a case of three components (viz. the two simple salts and water) which can form five phases: three solid (the two simple salts and the double salt), one liquid (the solution), and one gaseous (the aqueous vapour). But in the formation and decomposition of double salts in contact with solution, which ordinarily occurs in open vessels, there is absence of the possible gaseous phase; moreover, the change of solid phases into one another being accompanied with generally very little volume change, the influence of pressure on the equilibrium condition is practically negligible. These are the two properties characteristic of the special class of heterogeneous systems named by Van't Hoff *condensed systems*. Van't Hoff says: "In the investigation of the splitting up and of the formation of double salts, we follow out a special case of the study of so-called *condensed systems*, which exhibit peculiar transformation phenomena analogous to those of solidification and of fusion. The temperature named the *transition point*

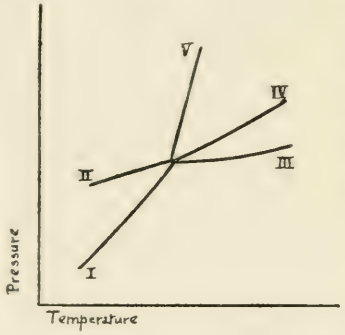
corresponds to the *melting point.* At the outset of his dealings with double salts, Van't Hoff had drawn attention to the fact that co-existence of the three phases double salt, one simple component, and solution, can occur only within a certain range of temperature, and that there is only one temperature (the transition point) at which there can be equilibrium between the solution and the three solid phases double salt and two simple salts.

Soon after, Roozeboom,[1] applying the principle and the terminology of the phase rule, recognised the transition point

TABLE III

THE QUINTUPLE POINT

in the non-variant system composed of three components (*e.g.* Na_2SO_4; $MgSO_4$; H_2O) present in five phases:



(1), (2), (3) Solid:
- Simple Salt MA . $p H_2O$, *e.g.* $Na_2SO_4 . 10H_2O$
- Simple Salt M'A . $p' H_2O$, *e.g.* $MgSO_4 . 7H_2O$
- Double Salt $M_m M'_n A_{m+n} . q H_2O$, *e.g.* $NaMg(SO_4)_2 . 4H_2O$

(4) Liquid: Saturated Solution of MA and M'A, *e.g.* Na_2SO_4 and $MgSO_4$

(5) Gaseous: Water Vapour

I. to V. are Vapour-pressure Curves for the Five Univariant Four-phase Systems.

I. for (1), (2), (3) and (5)
II. ,, (1), (2), (4) ,, (5)
III. ,, (2), (3), (4) ,, (5)
IV. ,, (1), (3), (4) ,, (5)
V. ,, (1), (2), (3) ,, (4)

of a double salt as the quintuple point of a non-variant system composed of three components present in five phases, this point being the intersection of curves for five *univariant systems*, each composed of four phases (Table III.).

Roozeboom's paper was followed in quick succession by the publication of much experimental and theoretical work on the lines by him initiated.[2] The greatest amount of attention was

[1] "Die Umwandlungstemperatur bei wasserhaltigen Doppelsalzen und ihre Löslichkeit," *Zs. physik. Chem.* 2, 1888, p. 513.

[2] Meyerhoffer, "Die gesättigten Lösungen der Verbindungen von Cuprichlorid mit Kaliumchlorid," *Zs. physik. Chem.* 5, 1890, p. 96; "Zur Kenntnis der Umwandlungsintervalle," *ibid.*, 9, 1892, p. 641. Vriens, "Dampfspannung des Kupferkaliumchlorids und seiner Lösungen," *ibid.*, 7, 1891, p. 194. Schreine-

devoted to the investigation of solubility relations. The temperature-concentration curves (Table IV.) for the three condensed 3-phase systems, viz.: (1) solution + simple salt MA + simple salt M′A, (2) solution + double salt + simple salt MA, (3) solution + double salt + simple salt M′A, are perfectly analogous to the corresponding temperature-pressure curves (Table III.), in that their intersection marks the transition point, *i.e.* the temperature at which the concentration of the different solutions is the same and at which the three solid phases, double salt and its two constituent simple salts, can co-exist. The graphic representation of these solubility relations has to take account of three variables, (i) the temperature and (ii and iii) the amount present in the saturated solution of each of the components MA and M′A. The most satisfactory manner of doing this is by means of space models with three rectangular axes,[1] though much may be accomplished by means of figures in a plane, such as those reproduced in Table IV. In the projection there adopted[2] the temperature axis is placed vertically, and the values found at different temperatures for the concentration of the two components MA and M′A (expressed as the number of molecules present in one hundred molecules of water) are represented by ordinates on both sides of the temperature axis. Hence two corresponding points such as G and G′, M and M′, etc., always belong together and refer to the same solution, whose concentration with respect to MA and M′A they represent for a special temperature.

The determination over a certain temperature range of such solubility curves, together with an extensive use of graphic methods for their representation, have made it possible to get complete knowledge of the equilibrium condition of the system

makers, "Gleichgewicht des Doppelsalzes von Jodblei und Jodkalium mit wässriger Lösung," *ibid.* 9, 1892, p. 57; and 10, 1892, p. 467; "Graphische Ableitungen aus den Lösungsisothermen eines Doppelsalzes und seiner Komponenten und mögliche Formen der Umwandlungscurve," *ibid.*, 11, 1893, pp. 55–110; "Theoretische und experimentelle Untersuchung über kryohydratische Temperaturen bei Systemen von zwei Salzen mit oder ohne Doppelsalzbildung," *ibid.*, 12, 1893, p. 73.

[1] Of such representations in space, that best known is the carnallite model, Van't Hoff and Meyerhoffer, *Zs. physik. Chem.* 30, 1899, pp. 86 *et seq.* See also Finlay, *The Phase Rule*, pp. 275 *et seq.*

[2] Koppel, "Die Bildungs- und Löslichkeitsverhältnisse analoger Doppelsalze," *Zs. physik. Chem.* 52, 1905, p. 385.

as dependent on the temperature and the concentration of the liquid phase, and also of the effect on the equilibrium condition of changes in temperature and concentration, the latter process being that termed *isothermal evaporation*. Thus the theoretical foundation was within a comparatively short time made so extensive and so firm, that in 1893 the relatively complex relations exhibited by the double potassium-magnesium sulphate could be elucidated with completeness and comparative ease.[1] It is impossible to give even in this paper a short exposition in due order and correct proportion of the theory of double-salt formation, as based on the phase rule. Hence, although recognising the unsatisfactory nature of the undertaking, all it is proposed to do is to say something about such special points or such special aspects as seem to present the greatest interest.

1. *The Experimental Determination of the Transition Point.*—The importance, theoretical and practical, attaching to knowledge of the exact temperature at which the double salt is formed or split, is such as to have led to the working out of a considerable number of experimental methods which it is possible to summarise and to classify into direct and indirect, as shown in Table V. The results obtained by the various methods exhibit not inconsiderable divergence (see values for astrakanite), and the suitability of the application of each of these varies with the special case under investigation. Short of saying a good deal about the technique of the different methods, it is not possible to give an adequate account of this aspect of the subject, and a brief mention of the principle on which they are based is all that can be attempted.

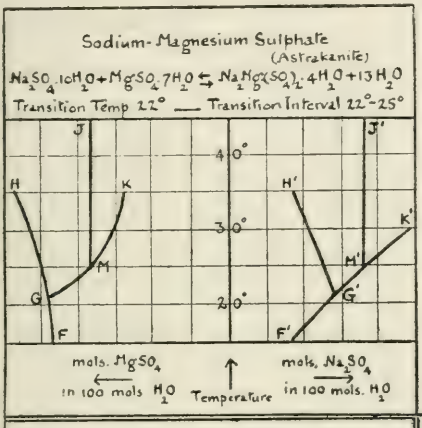
I. In those methods called *direct*, the transition point is found by actually ascertaining the temperature at which occur any of the changes—of volume, energy, appearance—which accompany the formation or the splitting of the double salt. Just as the change at 0° of the *solid* ice to the *liquid* water is accompanied by contraction in volume and absorption of heat, so the transformation of a double salt into the constituent simple salts is accompanied by contraction or expansion in volume (dilatometric method), absorption or evolution of heat (thermometric method), and sometimes also [method I. (iii)] by a change in colour, as in

[1] Van der Heide, "Doppelsalze von Kalium- und Magnesiumsulfat: Schönit und Kaliumastrakanit," *Zs. physik. Chem.* 12, 1893, p. 416.

TABLE IV

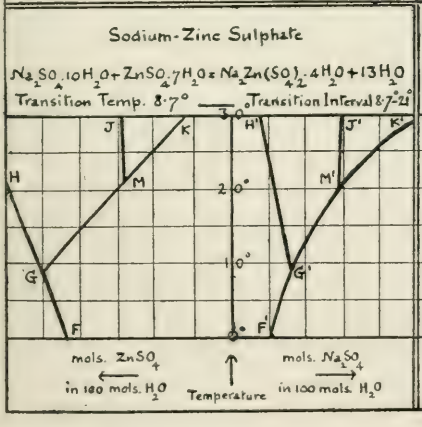
Solubility Curves for Double Salts and the Constituent Simple Salts

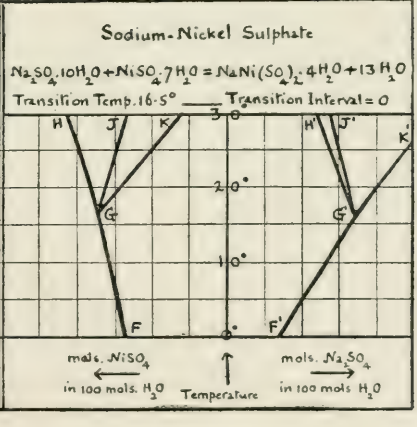
These temperature-concentration curves represent on horizontal axes at both sides of the vertical temperature axis the composition of solutions saturated with two sulphates and in equilibrium with two solid phases.



Curve:	Solid Phases:
FG and FG′	A mixture of the two *Simple Salts* $Na_2SO_4.10H_2O$ + $M''SO_4.7H_2O$
GK and G′K′	A mixture of the *Double Salt* and *Glauber's Salt* $Na_2M''(SO_4)_2.4H_2O$ + $Na_2SO_4.10H_2O$
GH and G′H′	A mixture of the *Double Salt* and the *Vitriol* $Na_2M''(SO_4)_2.4H_2O$ + $M''SO_4.7H_2O$
MJ and M′J′	*The pure Double Salt.*

GG′ is the Transition Point.
MM′ is the end point of the Transition Interval.





the case of the double acetate of copper and calcium (p. 137), or by a change in state of aggregation, as in the case of astrakanite (p. 142).

II. The methods named *indirect* are all based on the principle that at the transition point—that is, at the temperature which marks equilibrium between various states, when

$$m\mathrm{MA} + n\mathrm{M'A} \pm p\mathrm{H_2O} \gtrless \mathrm{M}_m\mathrm{M'}_n\mathrm{A}_{m+n} \pm p\mathrm{H_2O}$$

all the physical conditions for the two systems must be the

TABLE V

EXPERIMENTAL DETERMINATION OF THE TRANSITION POINT IN THE FORMATION AND SPLITTING UP OF **DOUBLE SALTS**

	Results *for Astrakanite*
I. Direct Methods	$\left\{\begin{array}{l}\mathrm{Na_2SO_4 \cdot 10H_2O} \\ \quad +\mathrm{MgSO_4 \cdot 7H_2O} \\ \quad\quad \downarrow\uparrow \\ \mathrm{Na_2Mg(SO_4)_2 \cdot 4H_2O} \\ \quad +13\mathrm{H_2O}\end{array}\right\}$
Based on finding the temperature at which occur the physical changes (in volume, heat of formation, colour, state of aggregation) accompanying the transformation of one system into the other.	
$m\mathrm{MA}+n\mathrm{M'A}\pm p\mathrm{H_2O} \gtrless \mathrm{M}_m\mathrm{M'}_n\mathrm{A}_{m+n}\mp p\mathrm{H_2O}$	
(i) Dilatometric	20·6 to 21·6
(ii) Thermometric	22·5 to 22·6
(iii) Observation of change in colour or state of aggregation	between 20 and 25
II. Indirect Methods	
Based on finding the temperature at which the equilibrium conditions are the same for the systems: Double Salt + Single Salt MA + Saturated Solution, Double Salt + Single Salt M'A + Saturated Solution, Single Salt MA + Single Salt M'A + Saturated Solution, *i.e.* when all three or any two of these saturated solutions have identical properties (*e.g.* composition, vapour pressure, etc.):	
(i) Solubility	22
(ii) Tensimetric	20·4
(iii) Electrical	20·7 to 20·9
(iv) Optical.	

same; such conditions are the composition, the vapour pressure, the electrical conductivity and electromotive force, and the refractive index of the *saturated solutions* of the following three combinations of two solid phases: (i) the two single salts, (ii) the double salt and the single salt MA, (iii) the double salt and the single salt M'A. Curves are constructed which represent for *two* or for all *three* of these combinations the variation of these properties with variation in temperature (Tables III.

and IV.), and the point of intersection of these curves gives the temperature sought.

2. *The Action of Water on the Pure Double Salt.*—Experience has shown that whilst some double salts, such as the alums, can at ordinary temperatures be recrystallised from water without decomposition, other salts cannot be so dissolved, and will only crystallise from a solution containing excess of one of the constituent simple salts. This decomposing action of water can be shown in a simple and striking manner in the case of the double lithium-copper chloride already referred to in this paper (p. 137). Even exposure to the moisture of ordinary air quite rapidly effects a colour change from the red of the double salt to the green of the constituent $CuCl_2 . 2H_2O$, a change which occurs instantaneously on moistening the garnet-red crystals with a drop of water; if however, instead of water, a drop of saturated solution of LiCl is used, the colour remains unaltered, showing the non-occurrence of decomposition due to the stability of the double salt in contact with a solution saturated with the more soluble of its constituents.

Other salts again, which like astrakanite dissolve at the transition point (25°) with decomposition and precipitation of the less soluble Na_2SO_4, dissolve at higher temperatures without decomposition. Examination of the curves of solubility (Table IV.) accounts for the above fact, that a pure double salt will at the transition point generally not dissolve without decomposition, and also for the few exceptions to this rule. The two simple salts generally differ in solubility, in consequence of which at the transition point the composition of the liquid phase in equilibrium with the three solid phases is not that of the double salt. Hence, if the double salt in excess is brought into contact with pure water, a greater amount of the more soluble simple salt will go into solution, and there will remain as solid phases the double salt and the less soluble simple salt. Thus, in the case of astrakanite, the component $MgSO_4$ is more soluble than the component Na_2SO_4; and hence, in bringing astrakanite into contact with water less in amount than that required for complete solution, more $MgSO_4$ will be dissolved, solid KCl remaining together with the excess of the double salt. From this it follows conversely that in order to obtain a double salt by crystallisation from a solution of the two components, it is necessary to have the more soluble of these present to the

greater amount required for the saturation of the solution, *e.g.* the copper-lithium chloride is obtained from a solution containing LiCl in considerable excess. But the relative solubilities of the systems involved vary with the temperature, and within the range of the possible existence of the double salt may be found a temperature at which the solution in contact with the double salt and the less soluble simple salt contains the two simple salts in the ratio in which they are present in the double salt; solution of the latter will then take place without decomposition.

The range of temperature between that at which the double salt can begin to be formed, and that at which it ceases to be decomposed by water, is called the *transition interval.* Thus, again taking the case of astrakanite, this salt is formed from the two simple salts at 22°. At a temperature just above this, solubility determinations show that the solution in contact with astrakanite and Glauber's salt contains more $MgSO_4$ than Na_2SO_4, but that at about 25° the two salts are present in equi-molecular quantities, and hence that the double salt will dissolve without decomposition. If the double salt is the stable form below the transition point, the transition interval will extend downwards to a lower temperature. The greater the difference in solubility of the two simple salts, the greater in general is the transition interval, though the temperature coefficient of the various solutions also influences the result. Consequently at ordinary pressures the transition interval is not always comprised within an attainable temperature range, and certain double salts, such as cupric-dipotassium chloride, lead-potassium iodide, can never be dissolved without decomposition. On the other hand, if the two salts have the same solubility at the transition point, a special case which is realised by *d* and *l* tartrates (Table II.), which combine to the racemate, or by sodium and nickel sulphates (Table IV.), the transition interval diminishes to zero, and the double salt dissolves without decomposition.

3. *The Connection between the Heat Effect accompanying the Transformation and the Temperature at which the Transformation occurs* (Table II.).—Some double salts, such as astrakanite, sodium-ammonium racemate, are formed as the temperature is raised, whilst for others, such as copper-calcium acetate, copper-dipotassium chloride, the reverse holds; this means that in some cases the form stable at ordinary temperature is the double

salt, whilst in others it is the mixture of simple salts. Again, some double salts, such as astrakanite and the double racemate, above mentioned, are formed with the splitting off of water, whilst for others the opposite is the case. Now the splitting off of water of crystallisation is generally a concomitant of heat absorption, the taking up of such water of heat evolution. And hence, in the case of the examples quoted—and it is the same in all others investigated hitherto—whenever the double salt is formed on raising the temperature, the transformation is accompanied by absorption of heat and splitting off of water, and *vice versa*, relations in perfect agreement with Le Chatelier's theorem. The heat evolution or heat absorption accompanying the formation of the double salt in each case acts in the contrary direction to the temperature change impressed on the system from without.

4. *Isothermal Evaporation.*—Much practical importance attaches to the consideration of this subject, *i.e.* to the effect produced by changing at constant temperature the concentration of a solution which contains two components capable of existing either as two simple salts or as a double salt. Study of the isothermal curves for temperatures without or within the transformation interval, makes it possible to foretell all the possible cases, and to arrive at rules which prove valuable guides in the practical processes of crystallisation.[1] To follow out this part of the study of double-salt formation, even in its general outlines, is beyond the scope of this paper; but one special case calls for mention, because of its great historical interest in another connection. From the point of view of the theory of double salts, this case is an instance of the difference in the results of an isothermal evaporation according as to whether this is carried out below or above the transition point. Pasteur's preparation of *l* rotatory tartaric acid is justly looked upon as the starting-point of the doctrine of stereo-isomerism, and his own highly dramatic account of the circumstances of this discovery constitutes one of the most attractive incidents in the history of science.[2] To Pasteur, crystallisation of a solution of optically inactive sodium-ammonium racemate yielded hemihedral crystals

[1] Van't Hoff, *Die Bildung und Spaltung von Doppelsalzen*, p. 8 *et seq.*; Finlay, *The Phase Rule*, chap. xvi.

[2] Pasteur, *Researches in Molecular Asymmetry*, Alembic Club Reprints No. 14 p. 20.

of two kinds, which when separated by a process of picking gave two sodium-ammonium tartrates, identical in all respects, except that whilst one was dextro-rotatory like the salt of ordinary tartaric acid, the other was lævo-rotatory to exactly the same amount. The failure of subsequent investigators to produce in crystallisation anything but the double racemate led to a thorough investigation of the whole subject,[1] and to the establishment of the fact that above 28° the double racemate crystallises out, whilst crystallisation below 28° yields a mixture of the two tartrates. Hence it has been said that Pasteur owed his discovery to the *happy chance*[2] that he carried out his evaporation below 28°.

5. *The Carnallite Problem.*—This is a case of technical importance. The potassium chloride used as the starting-point in the manufacture of the many potassium compounds of commercial importance (carbonate, hydrate, cyanide, nitrate, etc., etc.) is found native as the mineral *sylvite*, but by far the larger amount of that required is obtained from the mineral *carnallite*, $KCl.MgCl_2.6H_2O$. This salt has its transition point at 168°, when it breaks up into KCl, $MgCl_2.2H_2O$ and $4H_2O$. Owing to the great difference in solubility between the constituent chlorides, the transition interval is so extended that at ordinary pressure there is no temperature at which carnallite can be treated with water without partial decomposition and separation of the less soluble KCl as a solid phase. This is the principle underlying the old empirical process of the separation of the KCl, which is carried out as follows:

Treatment with water until a solution is obtained, which at 25° is saturated with carnallite, results in the separation of about five-sixths of the KCl in the solid form. The solution saturated for the double salt is evaporated, when carnallite crystallises out, and a solution is produced which for 1,000 molecules of H_2O contains 105 of $MgCl_2$ and 2 of KCl, and which therefore, for practical purposes, may be considered a solution of pure magnesium chloride. This solution is disposed

[1] Van't Hoff, *loc. cit.*, p. 82.

[2] Considering the popular meaning of the term "chance," one would wish to protest against its use in a connection such as the above, and to add some such parenthesis as: "chance, which in the matter of great scientific discoveries with unfailing discrimination and unbroken regularity selects for the recipient of its gifts only the truly great."

of as a waste product, whilst the carnallite that had separated from it is again worked up in the same manner as the original mineral.

Van't Hoff, in his Chicago lectures on "Physical Chemistry in the Service of the Sciences," has pointed out that application of the physico-chemical method to the carnallite problem reveals all the possible methods of splitting it up, methods of which the above process is only one amongst several. He proceeded to suggest another in which, instead of effecting the separation of the KCl by use of the solvent water and concentration of the solution obtained, temperature changes are resorted to in order to produce transformation, melting, and solidification. The process advocated (which has been patented by Van't Hoff's late colleague, Meyerhoffer) is as follows:

The carnallite is heated in closed vessels to 168°, the transition point, when three-fourths of the KCl separates out and is removed by filtration, the water split off retaining in solution all the $MgCl_2$ and one-fourth of the KCl. The liquid is then cooled to 115°, when carnallite crystallises out, leaving a liquid which is an almost pure melted hydrate of $MgCl_2$. The solid carnallite is separated by filtration and again worked up, whilst the liquid, which at ordinary temperatures becomes solid, constitutes the waste product.

The essential difference between the two methods—the empirical and that based on the application of physico-chemical methods—is that in the latter the $MgCl_2$ separated from the KCl is obtained, not as a mother liquor, but in the more convenient form of a solid.

The number of illustrative cases of the application of physico-chemical methods to the special problems encountered in the formation and decomposition of double salts could, of course, be greatly increased; but even the slightest respect for proportion makes it necessary to pass on to another and altogether different aspect of this subject.

The *physico-chemical* method, as developed on the basis of the phase rule, considers the existence of double salts in its dependence on temperature, pressure, and concentration, that is, on factors which it is possible to vary arbitrarily; and in this it differs essentially from the *chemical* method, whose object it must be to find an answer to the question: What relations must exist between two salts in order that—apart

from physical conditions—they may be capable of forming a double salt? Obviously we are dealing here with an affinity problem, and wish to ascertain something about properties fundamentally inherent in different special kinds of matter; we try to learn something about those conditions of double-salt formation over which, in entire difference from temperature, pressure, and concentration, we can exert no influence. In the absence of any means for measuring the affinity between two salts, what work has been done in this province has as yet barely gone beyond the classificatory; but even so, some interesting relations and useful applications have been established. It is proposed to give an account of some of these results, and to do so as shortly as possible under a somewhat arbitrary classification, dealing: firstly, with the work done that bears on the investigation of the relation between the elements which are capable of forming double salts, and the relation between those which are devoid of this power; secondly, with the investigations concerning the effect produced by the substitution of allied elements for one another on the power of forming double salts and on the properties of these salts; and thirdly, with the regularities found, or supposed to have been found, in the formulæ of double salts.

1. As the result of a very large number of experiments, it had long been recognised that an essential factor in the formation of double salts is a certain difference in the chemical character of the two metals. The sulphates of metals such as iron, zinc, magnesium, which crystallise with seven molecules of water, and which are termed *vitriols*, do not form double salts with one another, nor do the sulphates of the very similar elements potassium and cæsium; but there is great tendency to the formation of stable double salts between any one of the vitriols and any one alkaline sulphate. Moreover, the vitriols exhibit that characteristic of the existence of isomorphism which consists in the formation of a series of mixed crystals; and the more marked the chemical relationship, the greater is this tendency, as shown by the absence of any break in the series of the mixed crystals of magnesium and zinc. Similarly, the sulphates of potassium and cæsium are isomorphous. Though the general validity of this view concerning the difference in relationship required for the formation of mixed crystals and double salts respectively had been recognised before, it

remained for Retgers[1] to make it absolutely definite, and to show how it could be applied to argue backwards as to the kind and degree of relationship between different elements. He asserted that two simple salts which form a series of mixed crystals cannot form a double salt, and *vice versa*, that mixture and combination are mutually exclusive, and that this is true without reservation, without any exceptions whatever. Potassium and rubidium and ammonium form isomorphous mixtures, but do not form double salts; potassium and sodium are not isomorphous, but they do form double salts, such as for instance Seignette salt, the double potassium-sodium tartrate, or Scacchi's salt, the double sodium-ammonium racemate, which is a double salt in a double way (Table II.). Sulphates and selenates form isomorphous mixtures, but not double salts, whilst $NaNO_3$ combines with Na_2SO_4 to a double salt. Hence, on the basis of the absolute validity of this relationship, the fact that we know of a number of double salts between potassium and silver, but of none between sodium and silver, leads to the inference of a greater relationship between silver and sodium than between silver and potassium, and points to the probability of isomorphism between silver and sodium, which is proved by the existence of a series of mixed nitrates (and chlorates) of these two metals.

2. Moreover it follows, as a direct consequence of the recognition of chemical difference as the essential factor in the formation of double salts, that the *degree* of this difference ought to be of influence on the relative stability of the combinations produced. That this is actually the case is shown by the numerous instances in which it has been found that the most stable of all the alkali double salts are those of cæsium, itself the most electro-positive of all the metals; and in entire agreement with such incidental and unconnected observations is the result of an investigation on the double chlorides of ferric iron and the alkali metals, which well shows the connection between the stability of the double salt and the gradation in the electro-positive character of the alkali metals.[2] Between 0° and 60° NaCl could not be made to combine with $FeCl_3$; KCl yielded one double salt, viz. $FeCl_3 . 2KCl . H_2O$, whilst CsCl gave two

[1] Retgers, "Über chemische Verbindungen isomorpher Körper," *Zs. physik. Chem.* 15, 1894, p. 529.

[2] Hinrichsen and Sachsel, *loc. cit.*

such salts, viz. $FeCl_3 . 2CsCl . H_2O$ and $FeCl_3 . 3CsCl . H_2O$. It should be noted, however, that no numerical relations emerge from this research, though it allows us to trace the effect on the power of double-salt formation of the substitution of allied elements for one another.

In a later and very extensive investigation of the series of double salts formed between sodium sulphate and the class of sulphates called "vitriols," the realm of the quantitative is being approached.[1] The principle of this comparative study recalls that followed by Mr. Tutton in his extensive researches on the influence on crystalline form produced by the substitution in a series of salts of one isomorphous element for another. The special feature of this investigation of the class of double sulphates, $Na_2SO_4 . M''SO_4 . 4H_2O$ (Table IV.), lies in the fact that the determination of the transition point and the transition interval for each double salt made it possible to examine under strictly comparable conditions what is the influence of the properties of each special vitriol on the properties of the double salt into the composition of which it enters. Thus it could be shown that whenever the vitriol crystallises with seven molecules of water, as happens in the majority of cases (Mg, Zn, Fe″, Co, Ni), the double salt crystallises with four molecules; whilst copper and cadmium, whose stable hydrated sulphates contain five and one-half molecules of water respectively, give double salts containing only two molecules; and finally manganese, whose heptahydrate even at ordinary temperatures passes into a monohydrate, gives a tetrahydrated double salt, which however passes into the dihydrate below 40°, that is, at a temperature considerably lower than that of the dehydration of the other tetrahydrated double salts of the series. Connections have also been traced between the solubility of the vitriol and the extension of the transition interval, as well as between the electrical potential of the metal in the vitriol and the transition point of the double salt; but without the reproduction of a large number of numerical data these connections cannot be followed out satisfactorily. The results, moreover, are not very striking; and the chief reason for having said even thus much about this research is to show the method followed in a *chemical* study of double-salt formation.

3. The third and last of my arbitrary subdivisions of the

[1] Koppel, *loc. cit.*

chemical study of double salts deals with the generalisations concerning their formulæ. The record of achievement in this province is but meagre. The double halides are the class of double salts which have been most extensively used in this connection, the reason being that the total number known is very large, and that there is considerable diversity in the combining ratios of the constituents, as many as two or three different combinations between the same simple chlorides being known.[1] Remsen, from a tabulation of a large number of the double halides of the alkalies, has generalised to the effect that the number of halogen atoms due to the alkali metal is always less—or, at least, not greater—than that of the halogen atoms in the salt of the polyvalent metal, *e.g.* $CuCl_2 . 2KCl$, $FeCl_3 . 3CsCl$, etc. This generalisation, known as "Remsen's rule," seems, however, to be far from absolutely valid.[2]

Greater interest attaches to the theoretical speculations of Werner,[3] to the attempt made by him to find in the formulæ of double salts support for his view concerning the numerical values of the valency manifestations of the elementary atoms. Double salts have been amongst the chief of the representatives of so-called molecular combinations, a class of substances about which there has been a good deal of theoretical wrangling, and much discussion barren of practical results. Molecular compounds were, so to speak, merely invented, created in imagination in response to a desire for simplification of valency conceptions; and though furnished from time to time with supposed characteristics to serve as label, the attempt to differentiate molecular from atomic combinations cannot be considered to have proved successful. Werner is attempting to quite banish this conception of molecular compounds, of combinations specifically distinct from the atomic; and to do so he tries to remove by some other means the difficulty for the solution of which they had been created. Structural representation of the composition of double salts, of salts of complex radicles, of crystalline hydrates, etc., on the basis of atomistic combination, requires for

[1] Remsen, "Double Halogen Salts," *Amer. Chem. J.* 11, 1889, pp. 291 *et seq.*

[2] Wells, "Generalisations on Double Halogen Salts," *Amer. Chem. J.* 26, 1901, p. 389.

[3] Werner and Miolati, "Beiträge zur Konstitution anorganischer Verbindungen," *Zs. physik. Chem.* 12, 1893, pp. 35 *et seq.*; *Zs. anorg. Chem.* 3, 1893, p. 267 *et seq.*; 9, 1895, p. 382 *et seq.*

certain of the elements involved the manifestation of more combining units, *i.e.* of a higher valency than that deduced from their simpler combinations, which are the more numerous and the more stable. Now what Werner does is to conceive of two kinds of valency manifestations, the *chief* or *primary* valencies (Hauptvalenzen), which are effective in the compounds from whose composition the ordinary valency values are deduced, and the *subsidiary* or *secondary* valencies (Nebenvalenzen), which are different in nature from the first, as they bind entirely different radicles. The number of "subsidiary" valencies, like that of the "chief" valencies, is limited. He assumes that in compounds of a higher order the atom considered to form the nucleus can bind a definite number of other atoms, independent of the nature of these atoms, monovalent chlorine ranking alike with divalent oxygen. This number, which arises from the joint effect of "chief" and "subsidiary" valencies, is called the *co-ordination* number, and is defined as the limiting number which indicates how many atoms, independent of their valencies, are directly linked with one other special atom. The co-ordination number of an elementary atom can be deduced from the composition of its structurally known compounds, that is, from the maximum number of atoms, radicles, or groups linked with the elementary atom. In the majority of cases this number is found to be *six*, though *four* is of not infrequent occurrence. Thus when, in a simple salt, according to its ordinary valency value, the binding capacity of the metallic atom seems exhausted, it may still possess the power of certain atomic linkings whereby it participates in the building up of more complex molecules. Taking $FeCl_3$ as example, Werner points out how this salt, which by itself appears saturated, yet possesses the power of further combination with three apparently equally saturated molecules of KCl. [The argument is not invalidated by the fact that it has now been proved that the double salt is $FeCl_3 . 2KCl . H_2O$, and not, as Werner had supposed, $FeCl_3 . 3KCl$; the place of one molecule of KCl is taken by one of H_2O.] He says: "It is assumed that in the double salt $FeCl_3 . 3KCl$ all the six Cl atoms are linked with the Fe atom, that it contains the radicle $FeCl_6$, whose existence finds its explanation in the capacity of the iron to directly link six atoms—that is, in the co-ordination number six. Hence the co-ordination number is intended to represent that property of the atoms which makes

it possible to refer back the so-called 'molecular compounds' to actual linkings between quite definite atoms." An examination of the formulæ of the double salts dealt with in the course of this paper, will show that the majority of them support Werner's hypothesis; thus, for the double chloride of copper, the double sulphates of the astrakanite type, the co-ordination number is six. But at the same time there are undoubted exceptions, such as $FeCl_3 . 3CsCl . H_2O$, for which the co-ordination number would be seven; $CuCl_2 . LiCl . 2H_2O$, for which it would be five. It remains for future work to show how far these and other exceptions can be accounted for, and how far the hypothesis will prove deductively fruitful. At the present time it certainly marks an interesting attempt at simplification.

THE MECHANISM OF TISSUE RESPIRATION

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It is a fundamental property of all living organisms to be continually giving off carbon dioxide, and for all except certain bacteria and parasites to be continually absorbing oxygen. With these processes of respiration most of the so-called vital properties of protoplasm are so intimately bound up that, could we unravel them in their entirety, we should be not far from solving the mystery of life itself. How very remote we are from even a partial solution of the problem will be evident from the pages of this article, but we have at least made some progress towards it within recent years.

And firstly, how far are the carbon-dioxide-producing powers and oxygen-absorbing powers of living tissues dependent on one another? It would seem at first sight that they are but little related, for it was shown by Spallanzani in 1803, and since then by Johannes Müller and others, that animals such as the frog continue to exhale CO_2 even when entirely deprived of oxygen. In 1875 Pflüger repeated these observations, and he found that frogs, when kept in nitrogen containing no trace of oxygen, continued to give out CO_2 for several hours, at a rate but little inferior to that exhibited by frogs kept in air. Aubert made some direct comparisons of the CO_2 discharge of frogs kept in air or in nitrogen in a large glass cylinder over mercury. The experiments lasted four hours as a rule, and they were made at temperatures varying in different cases from 3·6° to

19·5° C. For purposes of comparison the results obtained have been grouped together and means taken.

Frogs kept in Air.			Frogs kept in Nitrogen.		
Number of Experiments.	Temperature.	CO_2 per hour per Kilogram.	Number of Experiments.	Temperature.	CO_2 per hour per Kilogram.
5	3·6° to 9·6°	24 c.c.	9	3·6° to 9·8°	24 c.c.
4	10·0° „ 14·5°	46 c.c.	3	11·3° „ 13·8°	43 c.c.
2	18·0° „ 18·5°	72 c.c.	8	15·0° „ 19·5°	70 c.c.

From these mean results we see that the CO_2 discharge of the frogs kept in nitrogen was practically as great as that of frogs kept in air, and that it was influenced to the same extent by rise of temperature. The lack of oxygen, though at first producing no obvious effect either in the CO_2 discharge or the vitality of the frogs, did so after a time, and the higher the temperature at which the experiment was carried out the sooner were the effects produced. The movements of the frogs became gradually feebler, and then ceased, though the heart continued to beat long after the nervous system was completely paralysed, and the CO_2 discharge continued to some extent even after cessation of heart-beat. At a temperature slightly above zero the frogs' movements continued as long as seven days. At 7° C. they continued for eight hours on an average, and at 12° for five hours; but at 21° they ceased in seventy-two minutes, and at 27° in twenty-five minutes. Though the CO_2 discharge persisted for longer than these periods, it slackened off very quickly at the higher temperatures, and so it was found that the *total* volume of CO_2 given off by the frogs was practically independent of the temperature. There seems to be a more or less fixed volume of CO_2 in the tissues of the frog, and this volume can either come off very slowly at a low temperature, or rapidly at a high one. Aubert did not attempt to fix the volume exactly, but he found that whether the frog were kept in nitrogen for 1,383 minutes at 7.9°, for 575 minutes at 11·8°, for 110 minutes at 22·8°, or for 35 minutes at 27·5°, it almost always gave off about 200 c.c. of CO_2 (reckoned per kilogram of body weight).

The CO_2 discharge in a nitrogen atmosphere has been measured also in the case of a snail (*Limax agrestis*) and a caterpillar (*Tenebrio molitor*). Thunberg, who carried out these

observations, found that with both these organisms the CO_2 discharge showed an immediate and progressive diminution from the moment the nitrogen was substituted for air. For instance, three snails kept in air at 19·6° gave out CO_2 at the rate of 322 c.c. per kilogram per hour. In nitrogen the rate of discharge dropped in successive half-hours to 202, 136, and 104 c.c., and then for the remaining five hours of the experiment to 80–90 c.c. Altogether 644 c.c. of CO_2 were given out in six and a half hours, whilst in another experiment at the same temperature no less than 1,088 c.c. were given out in the same time. These animals, therefore, contained a very much larger amount of separable CO_2 in their tissues than frogs do, for even at the end of these two experiments the CO_2 was still coming off at a fairly rapid rate. The caterpillars experimented with did not show quite so remarkable a result as this, but in six and a half hours at 17·5° they gave out CO_2 amounting to 446 c.c. per kilogram, and during a further twenty-two hours they gave out 532 c.c. more, or 978 c.c. in all.

In the case of warm-blooded animals it is obvious that exposure to an atmosphere of nitrogen would cause death and cessation of CO_2 discharge in a very few minutes. A liberation of CO_2 by the tissues does not absolutely cease, however, for the writer found that if an excised mammalian kidney be perfused with oxygenless saline it continues to give off CO_2 for some hours. This CO_2 rapidly dwindles down, and after an hour or two falls to 15–14 c.c. per hour per kilogram, but it was found that altogether nearly 100 c.c. per kilogram was discharged. The amount varied but little, whether the temperature of perfusion were 15°, 20°, or 28°, just as Aubert found it to vary but little in the frog. Whether mammalian tissues as a whole contain much less separable CO_2 than amphibian tissues it is impossible to say, for it is probable that the CO_2 stored up in the different organs of an animal varies considerably, being, for instance, large in muscle and small in nerve. But at least we may conclude that it is very much larger in the snail than in the caterpillar, and in the caterpillar than in the frog.

And by what processes is this CO_2 formed in the tissues in the absence of oxygen? Engelmann, Pflüger, and others suppose that the protoplasm of the cells, when exposed to oxygen, has the power of binding it and storing it up in an intramolecular

form, and that this store of combined oxygen confers upon the protoplasm its peculiar properties of instability and excitability. Further, it is supposed that once the store is exhausted, excitability is lost, or, conversely, that the persistence of excitability in living tissues after total deprivation of oxygen implies the existence within them of intramolecular oxygen. This hypothesis of intramolecular oxygen has been generally accepted, for it offers a reasonable explanation not only of the above-recorded facts concerning CO_2 production, but of many others. If the capacity of an organism or tissue for CO_2 production be a measure of its capacity for storing up intramolecular oxygen, then it follows that this capacity varies between very wide limits—*e.g.* as 100 to 1,200 or more. However, we know that at least some of the CO_2, and probably a great deal of it, arises in other ways than by intramolecular oxidation. The blood and lymph of mammals, and presumably also of the frog, contain 40 to 50 per cent. of their volume of loosely bound CO_2 Now we know that two-thirds or more of the total weight of an animal consists of water, only a small part of which is present in the blood and lymphatic vessels. Most of it is found within the cells, and this intracellular fluid, for aught we know to the contrary, may contain as much loosely bound CO_2 as the lymph of the lymphatics. If this were the case, then the total body fluids would contain about 300 c.c. of loosely bound CO_2 per kilogram, and if only two-thirds of it were liberated in frogs kept in an atmosphere of nitrogen, it would be unnecessary to assume that any of it was formed from intramolecular oxygen. Unfortunately no determinations have been made of the acidity or alkalinity of the blood and tissues of the frogs at the end of the experiments, or of the CO_2 set free from them on acidification, as compared with that from the blood and tissues of normal frogs. As we shall see later on, there can be very little doubt that acids are formed in the tissues under the conditions of the experiment, though we cannot say in what amount. The discharge of CO_2 by an organism kept in absence of oxygen is therefore no unequivocal proof of the existence of intramolecular oxygen in the tissues, though the estimations by the writer of the CO_2 discharged from mammalian kidneys on perfusion with oxygenless saline seem valid, and it was proved that in their case perfusion with dilute lactic acid provoked no liberation of CO_2.

Other proofs of the existence of intramolecular oxygen are mostly of the type first brought forward by Engelmann in 1868. Engelmann kept ciliated epithelial cells in an atmosphere of hydrogen till all movement ceased, and then added sufficient oxygen to start the movements again. On replacing this mixed atmosphere with pure hydrogen, it was found that cessation of ciliary movement ensued earlier than on the first occasion. The longer the cells were kept in oxygen, however, the longer time did the ciliary movements persist in the hydrogen, so it looked as if the cells had been able to store up a supply of intramolecular oxygen on which they could gradually draw. Again, Öhrwall found that if the flow of perfusion liquid through an excised frog's heart (kept in a hydrogen atmosphere or in salt solution) were stopped, the heart ceased beating in one to three hours. If the perfusion were resumed, the heart speedily began to beat again; but if the perfusion were now stopped it ceased beating in four to twenty-five minutes—*i.e.* in a very much shorter time than previously.

Experiments of a similar nature have been made by Bernard upon fish, and by Frohlich and by Baglioni upon nerve; but, as Winterstein points out, none of these results afford convincing proof of the existence of intramolecular oxygen. Winterstein, indeed, denies the existence of this oxygen, and he supposes that the loss of excitability produced by deprivation of oxygen is due to the accumulation of toxic products of fatigue. In the presence of free oxygen, these products are gradually oxidised and rendered harmless, and so it follows that if the inexcitable tissue be exposed to oxygen for only a short time, but small amounts of the toxic products are oxidised, and so a second deprivation of oxygen induces a much speedier loss of excitability than the original deprivation did. This explanation might be held to be negatived by an interesting experiment of Verworn's, in which a frog was perfused with oxygen-free salt solution. Strychnine was added to the saline so as to heighten the excitability of the cord, and for some minutes each stimulation of the skin resulted in a succession of brief reflex muscular contractions. If during this initial period the flow of oxygenless saline were stopped, the cord speedily lost its reflex excitability; but if the flow were resumed again, it was found that the excitability might return for a short time. It then disappeared permanently so long as the flow of oxygenless saline was continued,

and could only be brought back again by the substitution of oxygenated saline. Verworn thought that the first brief recovery of excitability was due to the oxygenless saline washing away the products of fatigue from the tissues, and that the tissues still retained a small amount of intramolecular oxygen. Once this was used up, however, no further recovery of excitability was possible until the tissues were supplied with more oxygen from external sources.

Even this experimental proof, ingenious at it is, does not finally prove the point at issue, for it is possible that some of the toxic products of fatigue are soluble, and that the removal of these products by oxygenless saline leads to some recovery of excitability. Other of these products may be insoluble, and only be removable after oxidation by free oxygen.

Still another type of argument in support of the doctrine of intramolecular oxygen is derived from a study of the respiratory quotients yielded by an animal before, during, and after deprivation of oxygen. For instance, Thunberg observed that caterpillars kept in air gave a quotient of ·81. On substituting an atmosphere containing 99·5 per cent. of nitrogen and ·5 per cent. of oxygen, their oxygen intake fell to about a tenth of its previous value, whilst their CO_2 output fell to about a fifth—*i.e.* the respiratory quotient became double the normal. On returning the caterpillars to air again, the CO_2 output increased considerably—though it was never more than half its original value—but the oxygen intake increased in a still greater proportion, and respiratory quotients of ·64, ·67 and ·70 were obtained in successive half-hour intervals. Relative to their CO_2 output, the caterpillars were therefore absorbing oxygen at a greater rate than usual, apparently in order to replenish the stock of intramolecular oxygen they had drawn upon during the nitrogen period. The writer obtained similar results with mammalian kidneys, though he found that the extra volume of oxygen absorbed on substituting oxygenated saline for the previous oxygenless perfusion liquid was only a third to a half as great as would be expected from the output of CO_2 during the oxygenless period.

But this type of result can likewise be explained on the hypothesis of fatigue products. Such products, if insoluble, may collect in the tissues during the oxygenless period, and require

an extra amount of oxygen for their complete removal during the subsequent oxygen period. To take a concrete instance, one suggested by Winterstein, the decomposition of a carbohydrate molecule may take place in the following two stages:

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$
$$2C_2H_5O + 6O_2 = 6H_2O + 4CO_2.$$

The change represented by the first equation results in the formation of a third of the total CO_2 output, though no oxygen is drawn upon whatever; whilst according to the second equation the complete oxidation of the alcohol— a "fatigue product" produced by the first decomposition—requires 6 volumes of oxygen for every 4 volumes of CO_2 formed, or yields a respiratory quotient of ·67.

Arguing against the somewhat inconclusive evidence of the existence of intramolecular oxygen, Winterstein adopts the views long since suggested by Detmer, that the primary source of the energy developed in living organisms is not of an oxidative nature, but consists rather in the disintegration of highly complex labile compounds, the decomposition products of which subsequently undergo oxidation. That under certain conditions vital energy *can* be developed in the absence of contemporary oxidation is undoubted, for we know that certain bacteria, as *B. anthracis* and *B. tetani*, live and flourish best under completely anærobic conditions. It seems very probable, also, that some of the energy liberated in ærobic organisms may be of similar origin, but we cannot for a moment imagine that all the energy is developed in exactly the same way. Much, perhaps most of it, is in all probability formed as the result of contemporary oxidation. If this is the case, then a supply of immediately available intramolecular oxygen is essential, and arguing from the weight of evidence, which is admittedly not quite conclusive, we should provisionally adopt the doctrine of its existence.

We must now pass on to a discussion of what is actually known as to the processes of oxidation in the tissues. Mention has frequently been made in the above pages of the collection of fatigue products in the tissues, which subsequently undergo further oxidation; but are such products matters of fact, or mere figments of fancy? Some of the most interesting evidence

bearing upon this question is that obtained by Fletcher in his studies upon the respiration of excised frogs' muscles. A slow current of air or other gas was drawn over the muscle or muscles placed in a suitable chamber, and the CO_2 given off absorbed by baryta and estimated by titration. On tetanising the muscles kept in air or in nitrogen for periods of 10 to 30 minutes, little if any increase in the CO_2 output was observed, provided that the muscles did not, as the result of fatigue, pass into a state of rigor mortis. If this was the case, then the increase of CO_2 might be considerable, and the increase observed by Hermann on tetanisation of muscle is probably to be ascribed to this cause. When, on the other hand, the muscles were kept in a current of pure oxygen, not only was the output of CO_2 during the resting stage increased, but on tetanisation it became more than doubled in amount. For instance, five gastrocnemii in a current of nitrogen gave out ·05 c.c. of CO_2 per half-hour during rest, and ·07 c.c. during tetanisation. Five other gastrocnemii (taken from the opposite limbs of the same five frogs), when kept in oxygen, had at first an output of ·08 c.c. of CO_2 during rest, and ·14 c.c. during activity, and later on an output of ·095 c.c. during rest and ·23 c.c. during activity. It was also noted by Fletcher that fatigue came on much more rapidly in muscles kept in air or nitrogen than in those kept in oxygen, and that fatigued muscles, if allowed to rest in oxygen, recovered much more rapidly and perfectly than if allowed to rest in nitrogen. Still again, he noticed that muscles kept in oxygen never passed into rigor mortis at all. They survived many hours—sometimes as much as fifty hours—longer than muscles kept in nitrogen or air, and then underwent a gradual lengthening instead of shortening. These and other results are most readily explained by supposing that during the resting stage, and still more during activity, there is a continual breaking down of complex molecules in the muscle substance into simpler ones, but that these simpler molecules do not reach their final CO_2 stage except in the presence of free (or intramolecular) oxygen, and that within certain limits the more plentiful the supply of oxygen the more complete and rapid is the oxidation. In an excised gastrocnemius oxygen can only reach the tissue cells by diffusion from the outside, and hence the inner layers of a muscle kept in oxygen would be very much more adequately oxygenated than those of a muscle kept in air.

As to the intermediate products of muscle activity, there is not much definite information, but undoubtedly sarcolactic acid is one of them. The recent results of Fletcher and Hopkins show that absolutely fresh muscle (of the frog) contains only traces of lactic acid (·015 per cent. or possibly less). Fatigued muscle, on the other hand, may contain a considerable amount of lactic acid (·11 to ·21 per cent.), and rigored muscle still more (·24 to ·40 per cent.). The amount of lactic acid any given muscle can develop seems practically constant, whether it be formed suddenly by exposing the muscle to a temperature of 45°, or more slowly by tetanising the muscle when placed in an atmosphere of hydrogen or oxygen, and finally inducing heat rigor. It is formed gradually in resting excised muscle, if this muscle be kept in an atmosphere of hydrogen or nitrogen, *i.e.* under anærobic conditions, but if kept in air it is formed only very slowly, and if kept in oxygen it is not formed at all. On the contrary, as might be expected from Fletcher's results on the restorative power of oxygen on irritability, if muscles in which a considerable lactic acid formation has been induced by tetanistion are placed in oxygen, a good deal of this lactic acid disappears. Such disappearance of lactic acid was found to be dependent on the integrity of the muscle, no diminution showing itself in chopped-up muscle.

Other evidence of lactic acid formation was obtained by Spiro, who found that blood collected from the carotid of a dog in which the muscles of the hind limbs had been tetanised for an hour and a quarter showed quite an appreciable increase of sarcolactic acid. Again, Max von Frey and Gruber found that if an isolated muscle of the dog were perfused with an artificial circulation of blood, and were made to contract, there was a considerable increase in the absorption of oxygen, but a by no means corresponding increase in the output of CO_2. There was a striking rise in the sarcolactic acid in the blood, however, which more than accounted for the deficiency of CO_2. Presumably this acid was not oxidised further in the tissues owing to the abnormal conditions of experiment, but it is possible, as suggested by Von Frey, that even in the intact living animal the lactic acid formed in the muscles may be to some extent oxidised elsewhere. In support of this view may be quoted the observations of Meyer, who found that the diminution of CO_2 production induced by arsenic and phosphorus poisoning was accompanied

by an accumulation of lactic acid, especially in the liver: also those of Araki, who showed that lactic acid appeared in the urine of animals poisoned by carbon monoxide, phosphorus, or arsenic.

The writer has obtained further evidence of the incompleteness of tissue oxidation under abnormal conditions by experiments upon excised mammalian kidneys. When these organs are perfused with oxygenated Ringer's solution for ten or twelve hours, their vitality gradually diminishes, but the processes of tissue oxidation, though gradually becoming feebler in magnitude, do not lack completeness, and the respiratory quotient remains practically constant at about ·85. Kidneys which previous to perfusion have been heated for half an hour to 50° to 60°, or frozen solid and thawed, or been kept in a moist chamber for three to nine days after excision, or poisoned by hydrocyanic acid, likewise show considerable diminution in their gaseous metabolism, but the processes of tissue oxidation still remain complete, and the respiratory quotient keeps at about ·85. When, on the other hand, a kidney was perfused with oxygenated saline solution containing ·06 to ·10 per cent. of lactic acid, or ·005 to ·025 per cent. of free ammonia, its oxygen-absorption power rapidly diminished, but its CO_2-producing power fell off more rapidly still, so that after four to eight hours of perfusion its respiratory quotient dropped to ·46–·36, *i.e.* to about half the normal.

All the experimental evidence available tends to show, therefore, that the processes of tissue oxidation take place in more than one stage, and that the earlier stages are more readily accomplished than the final stage which leads to the production of free CO_2. How numerous or complex these stages are we have no definite means of knowing, but it is by no means improbable that they are of a comparatively simple nature. It is well known that increased functional activity of muscles or other organs, though it may lead to a tenfold increase of oxygen absorption and CO_2 production, has no effect whatever upon their nitrogenous metabolism. Hence Verworn supposes that only non-nitrogenous groupings or side chains in the "biogen molecules" of the protoplasm are concerned in the ordinary processes of tissue respiration, and he suggests that these side chains may be carbohydrate groupings of an aldehyde character. Also he supposes that intramolecular oxygen is stored up in the

biogens in the form of an NO_2 grouping attached to a benzene ring, and that one atom of oxygen from the NO_2 oxidises the $-CHO$ or $-CHOH$ groups of the carbohydrate molecule to CO_2 and water.

The hypothesis that the respiratory processes of tissues are carried out by non-nitrogenous side chains is borne out by some curious and unexpected results obtained by the writer. It was found that when recently excised mammalian kidneys were perfused with saline solution, the protoplasm of the tissues was in a state of such extreme instability that it was liable to undergo sudden disintegration, whereby a good deal of proteid, proteid decomposition products and intracellular ferments (such as erepsin) passed out into solution. In various experiments carried out under normal conditions, from 9 to 17 per cent. of the total proteids present in the kidney tissues were washed out during the course of an eleven-hour perfusion, and yet the respiratory powers of these kidneys were just as great as those of other kidneys in which there had been little or no tissue disintegration.

The carbohydrate-like nature of the side chains receives support from the above-mentioned facts concerning lactic acid formation in the tissues. We know that sugars are converted into lactic acid not only by the action of various bacteria, but also by intracellular enzymes such as the lactacidase enzyme of the expressed juice of yeast (Buchner and Meisenheimer). Such lactic acid formation may be a normal stage in the oxidation of the carbohydrate-like chains to CO_2, or it may be an exceptional one, occurring but seldom unless the conditions of oxidation in the tissues are deficient. If the $-CHOH$ groupings of the carbohydrate side chains are oxidised one at a time, they would presumably pass through an aldehyde stage first. That an aldehyde stage does occur at some point or other in the course of tissue respiration, seems to be borne out by some observations of the writer. Thus it was found that if an excised mammalian kidney were perfused with saline containing ·1 to ·2 per cent. of hydrocyanic acid, its gaseous metabolism, when estimated shortly after in the usual way by perfusion with normal oxygenated saline, was at a minimum at first, but that it steadily increased for the next eleven hours. During the perfusion, small quantities of HCN broke away from the tissues, and hence it was surmised that this HCN was bound up to $-CHO$ groups so as to form

– CH(CN)OH groups, and that as long as it was so bound the tissues lost their respiratory powers. Similar results were obtained by perfusing kidneys with saline containing ·2 per cent. of acid sodium sulphite. On subsequent perfusion with oxygenated saline the gaseous metabolism of the tissues rapidly increased, and after ten hours became more than double its original value. This result was presumably dependent on the well-known powers possessed by aldehydes of combining with acid sulphites.

Small as is our knowledge concerning the possible or probable course of oxidation of carbohydrate groupings in the biogen molecules, that concerning fats is still less—in fact, it is non-existent. In the fats we have chains of $-CH_2$ groupings, instead of –CHOH groupings, and probably these groupings are oxidised gradually, one by one, as we assumed the –CHOH groupings to be. Proteids may undergo oxidation in more or less the same way, their constituent amino-acid groupings first splitting off ammonia, and then undergoing oxidation in the same way as the fats.

Still one more question remains for our discussion—viz. that concerning the relation of tissue respiration to oxygen tension. We saw that caterpillars kept in an atmosphere of nitrogen containing only ·5 per cent. of oxygen absorbed only a tenth as much oxygen as when they were kept in air, but what would be the extent of oxygen absorption with atmospheres of intermediate composition? To determine this, Thunberg has made a large number of estimations at various oxygen tensions, and some of his mean results are given in the table:

Percentage of Oxygen in Atmosphere.	Mean Oxygen Absorption.	
	Caterpillar (*Tenebrio*).	Snail (*Limax*).
1	46	—
5	83	46
10	93	73
21	100	100
50	—	117
90	—	122

In each series the absorption power in air is taken as 100, and we see that in the case of the caterpillar the oxygen absorbed

increased with the tension, rapidly at first, and then more and more slowly. In all probability the caterpillars kept in air were absorbing oxygen almost at their maximum rate. In the snail, however, the affinity of the tissues for oxygen was so small that from the 5 per cent. mixture only half as much oxygen was absorbed as in the case of the caterpillar, and the maximum absorption rate was not attained in an atmosphere containing 50 per cent. of oxygen. In mammals it would appear that the affinity of the tissues for oxygen is very much greater than in these lowly organisms, for the maximum absorption rate is attained at very much smaller tensions. More than a century ago Seguin and Lavoisier showed that the oxygen absorption was, within certain limits, independent of the oxygen content of the atmosphere breathed. This question has since been repeatedly examined by other observers, and the general consensus of opinion upholds the original doctrine. Löwy, for instance, has found that the oxygen absorption is constant whether the oxygen in the atmosphere breathed be increased to more than double the normal, or diminished to less than half the normal. Of course it does not follow that the tension of oxygen in the blood reaching the tissues varies to the same degree as that in the air respired, but there can be no doubt that it can vary within wide limits without affecting the gaseous metabolism of the tissues. In the case of excised mammalian kidneys, the writer found that at a temperature of 20° the oxygen absorption diminished if the tension of oxygen in the einals used for perfusion fell below half atmospheric pressure, but otherwise it was unaffected.

Pflüger endeavoured to account for this constancy in the oxygen-absorptive capacity of an animal by supposing that through the metabolism of the cells in any given moment only a fixed number of oxygen-binding affinities are liberated, and hence that any further increase in the available oxygen above that required by them is without influence. On this view the tissues must have more oxygen at their command than they can use up, or be always saturated to their full capacity with intramolecular oxygen. Thunberg explains his results by supposing that the restitution of the intramolecular oxygen absorbed in the metabolism is a somewhat slow process, and that the greater the oxygen tension the quicker the restitution, and the greater the supply of intramolecular oxygen available

the more rapid the metabolism. This view can be combined with that of Pflüger, only we must suppose that in the tissues of mammals the restitution of intramolecular oxygen occurs more rapidly and at lower tensions than in those of lower animals. It is very probable that the great difference observed is due chiefly to the difference of temperature. At 37° the processes connected with the absorption and chemical combination of the intramolecular oxygen would doubtless occur more rapidly than at 19°, which is the temperature at which most of Thunberg's experiments were carried out.

Into the question of oxygen absorption in relation to functional activity I do not propose to inquire. We know, for instance, that an active submaxillary gland absorbs oxygen more than three times as fast as a resting gland (Barcroft), an active kidney more than twice as fast as a resting kidney (Barcroft and Brodie), but we are absolutely ignorant of the inner mechanism by which a nervous or chemical stimulus induces an increase of tissue metabolism. In all probability the chemical changes occurring in the tissues of the active glands are of the same character as those in the resting glands, only they take place at a more rapid rate, just as they take place more rapidly at a higher than at a lower temperature.

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